



biogen idec

**The Intrinsic Stability of Antibodies
Based on Experiment, Design and
Prediction**

Stephen Demarest

Engineering Stability into Antibody Domains

- **For Engineerability/Function**
- **Antibody domain constructs, i.e. scFvs, intrabodies, etc., often suffer from stability problems**
- **Expression in non-mammalian hosts**
- **For Solubility/half-life**
- **For Manufacturing and Purification**

What could be
worse than
low expressing
and aggregated
antibodies?

Being eaten
by a walrus?

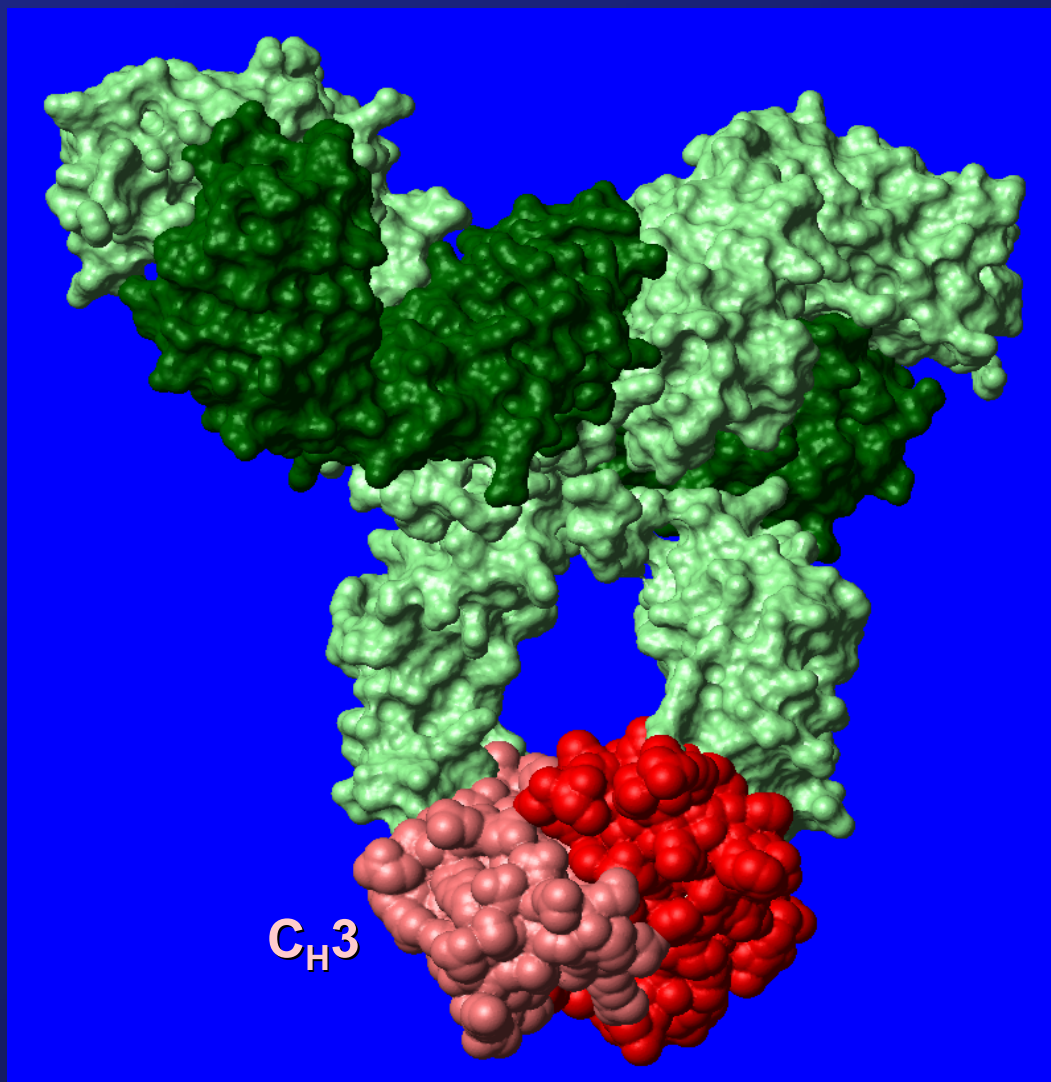


Sequence Basis for Stability Designs

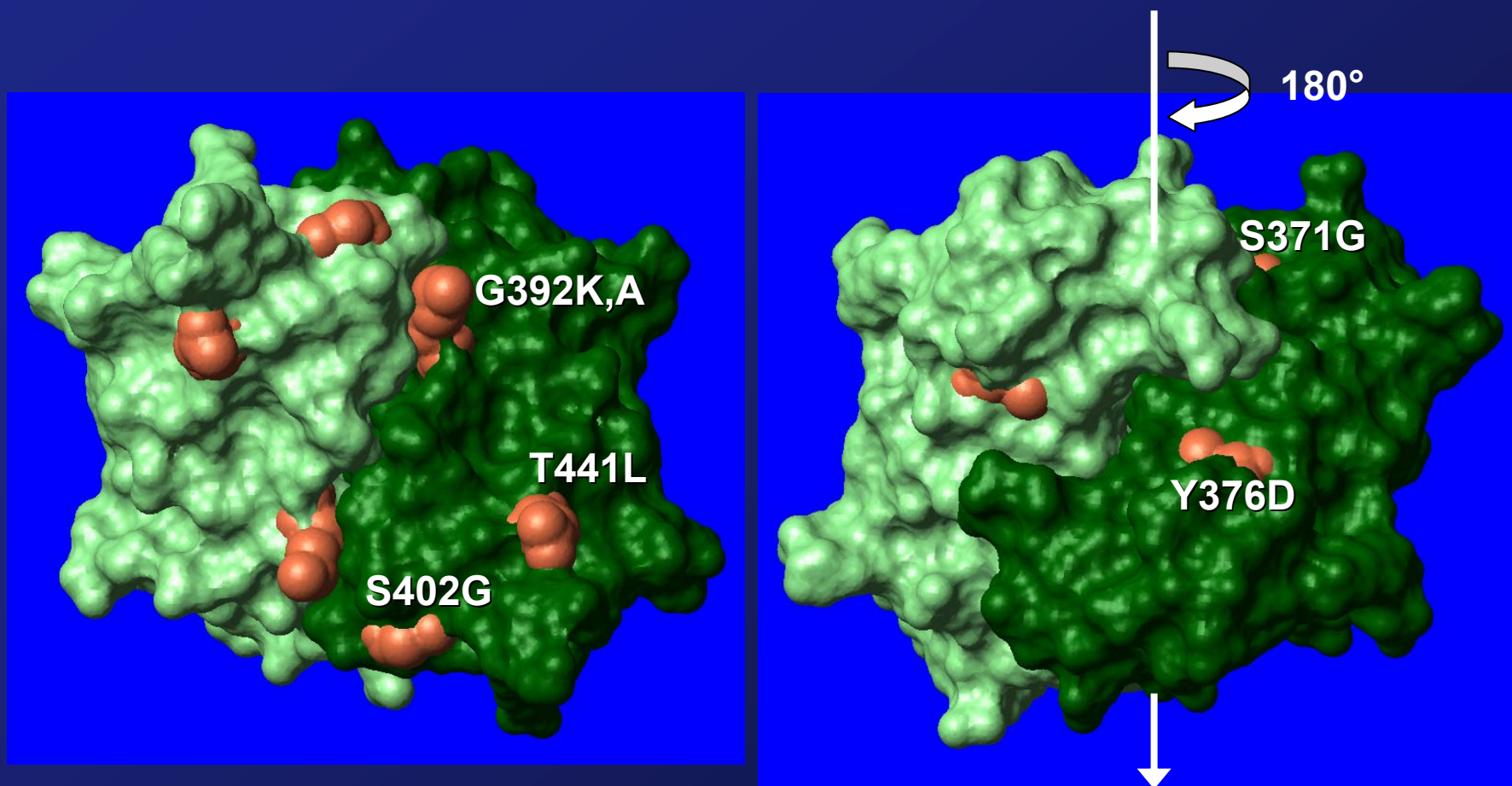
- **Many established methods – even for antibodies.**
- **Sequence information can provide rapid, unbiased basis for designs.**
- **Recent explosion in the genome sequencing of all organisms.**

rattusnorvegicusIgG1	ITLTPKVT CVVVDISQDDPEVH --FSWFVDDVEVHTAQ--TRPPEEQFNS
rattusnorvegicusIgG2a	ITLTPKVT CVVVDISQNDPEVR --FSWFIDDVEVHTAQ--THAPEKQSNS
musmusculusIgG3	ISLTPKVT CVVVDVSEDDPDVH --VSWFVDNKEVHTAW--TQPREAQYNS
CricetulusmigratoriusIgG	ISLTPKIT CVVVDVSEEEPDVQ --FNWYVNNVEDKTAQ--TETRQRQYNS
OryctolagusuniculusIgG	ISRTPEVT CVVVDVSQDDPEVQ --FTWYINNEQVVRTAR--PPLREQQFNS
SylvilagusunicularisIgG	ISRTPEVT CVVVDVSQDDPEVQ --VTWYINNEQVHTAR--PPLQEQQFNS
LepuscalifornicusIgG	ISLTPEVT CVVVDVSQDDPEVQ --FTWYINNKQVHTAR--PPLREQQFNS
sheepIgGC	ISGTPEVT CVVVDVGQDDPEVQ --FSWFVDNVEVVRTAR--TKPREEQFNS
ovisariesIgG1	ISGTPEVT CVVVDVGQDDPEVQ --FSWFVDNVEVVRTAR--TKPREEQFNS
boTaurusIgG1	ISGTPEVT CVVVDVGHHDDPEVK --FSWFVDDVEV NTAT --TKPREEQFNS
boTaurusIgG3	ISGTPEVT CVVVDVGQDDPEVQ --FSWFVDDVEVHTAR--TKPREEQFNS
humanIgG1	ISRTPEVT CVVVDVSHEDPEVK --FNWYVDGVEVHNAK--TKPREEQYNS
PantroglodytesIgG	ISRTPEVT CVVVDVSHEDPEVK --FNWYVDGVEVHNAK--TKPREEQYNS
humanIgG2	ISRTPEVT CVVVDVSHEDPEVQ --FNWYVDGVEVHNAK--TKPREEQFNS
macacafascicularisIgG1	ISRTPEVT CVVVDVSQEDPDVK --FNWYVNGAEVHHAQ--TKPRETQYNS
macacamulattaIgG2	ISRTPEVT CVVVDVSQEEPDK --FNWYVDGVEVHNAQ--TKPREEQFNS
susscrofaIgG2a	ISRT PQVT CVVVDVSQENPEVQ --FSWYVDGVEVHTAQ--TRPKEEQFNS
susscrofaIgG1	ISQTPEVT CVVVDVSKEHAEVQ --FSWYVDGVEVHTAE--TRPKEEQFNS
lamaglamaIgG1	ISGRPEVT CVVVDVGKEDPEVN --FNWYIDGVEVVRTAN--TKPKEEQFNS
CamelusdromedariesIgG	ISGRPEVT CVVVDVGQEDPEVN --FNWYIDGVEVVRTAN--TKPKEEQFNS
canisfamiliarisIgGC	TARTPTVT CVVVDLDPENPEVQ --ISWFVDSKQVQTAN--TQPREEQSNG
canisfamiliarisIgGA	ITRTPEVT CVVLDLGREDPEVQ --ISWFVDGKEVHTAK--TQSREQQFNG
mustelavisonIgG	ISRTPEVT CMVVDL --EDPEVQ--ISWFVDNQEMHTAK--TNSREQQFNS
feliscatusNRSDIgG1	ISRTPEVT CLVVDLGPDDSDVQ --ITWFVDNTQVYTAK--TSPREEQFNS
equuscaballusIgG1	ITRTPEVT CVVVDVSQENPDVK --FNWYMDGVEVVRTAT--TRPKEEQFNS
equuscaballusIgG2	ISRT PVVT CVVVNLSDQYPDVQ --FSWYVDNTEVHSAI--TKQREAQFNS
OrnithorhynchusIgG1	VAGTPKVT CVVVDLGFEDKDES PVVTWYQGDKEPKTRMLEPPPKEQRNG
TachyglossusaculeatusIgG	VTGTPKVT CVVVDLGFEDKDEN PVVTWYQGDKEPKSGSVERRRKEQRNG
MonodelphisdomesticaIgG	LSRSPKVT CMVVDVS -DASGVQ--ITWFKGEEEVSSPK

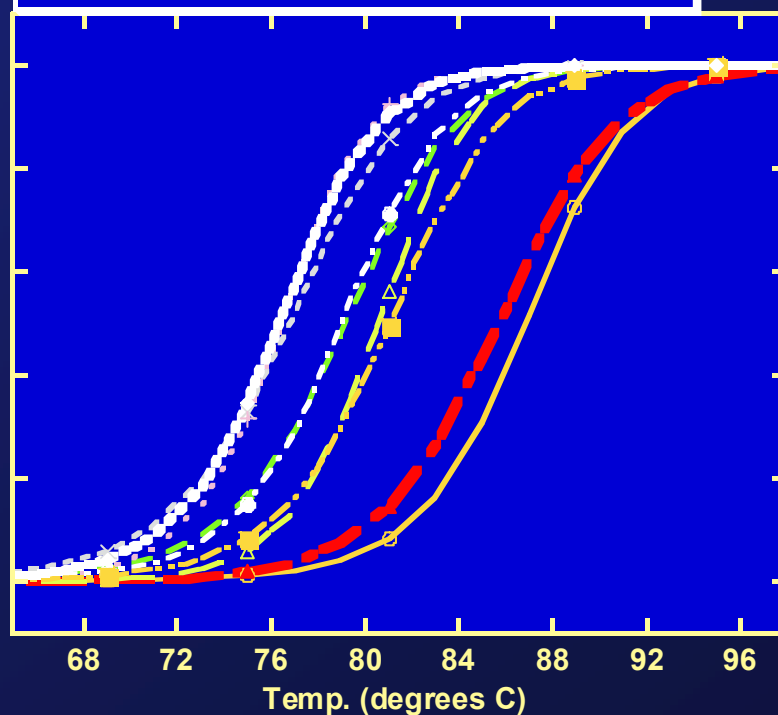
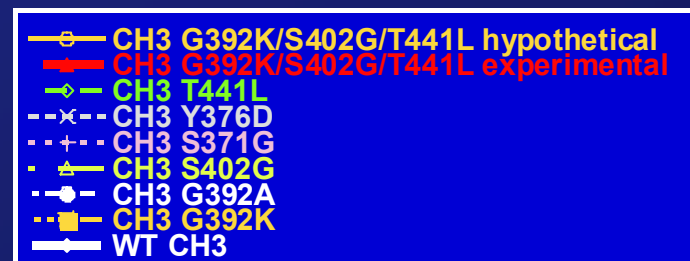
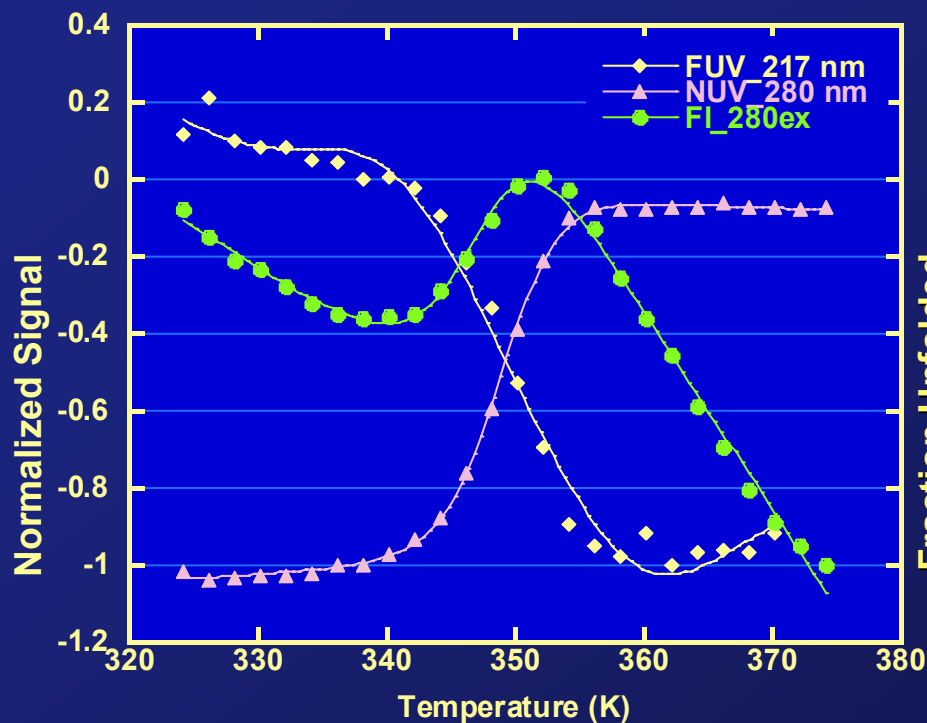
C_H3 Chosen as Initial Domain for Engineering



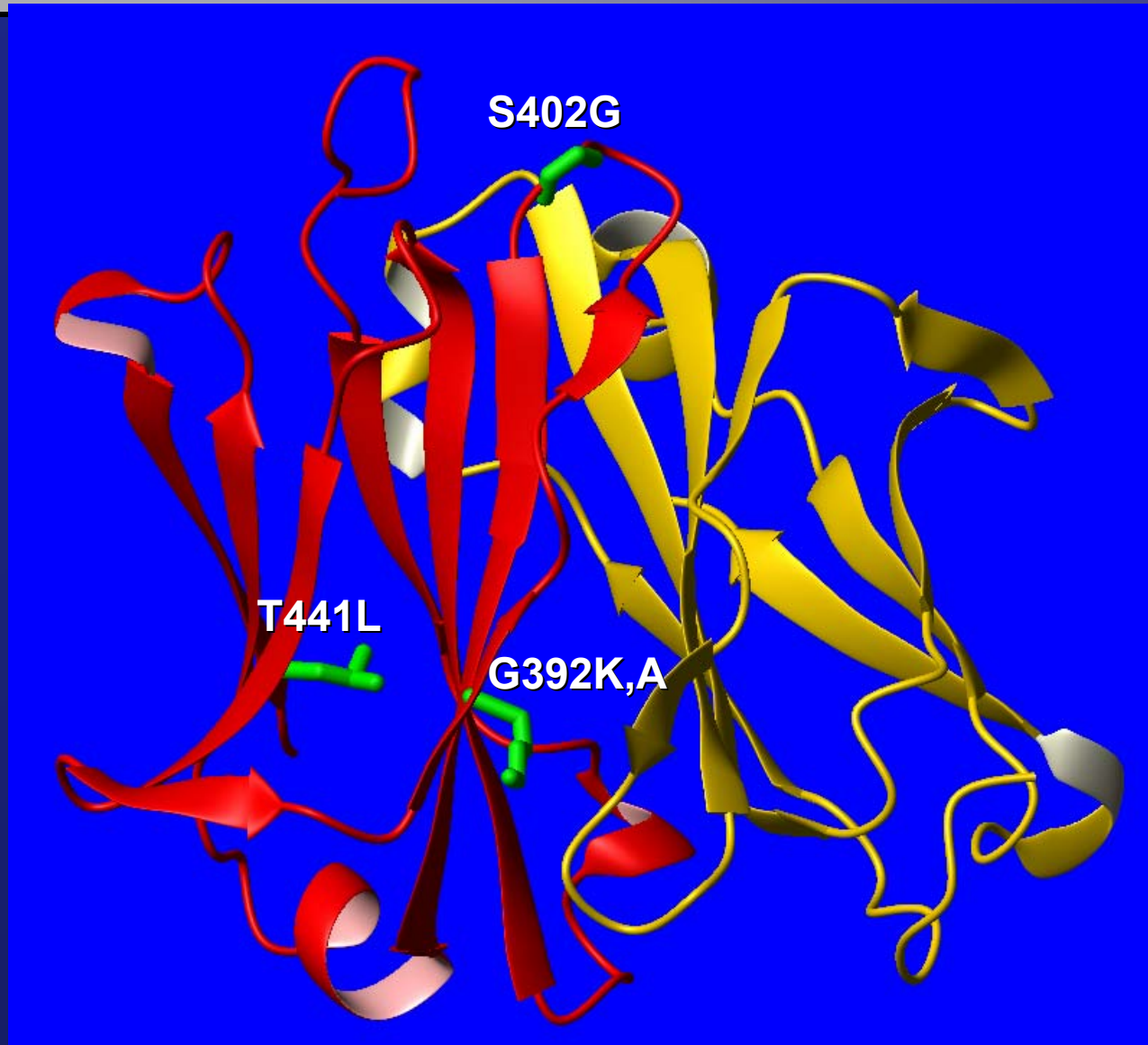
Potential Weaknesses within the C_H3 Domain



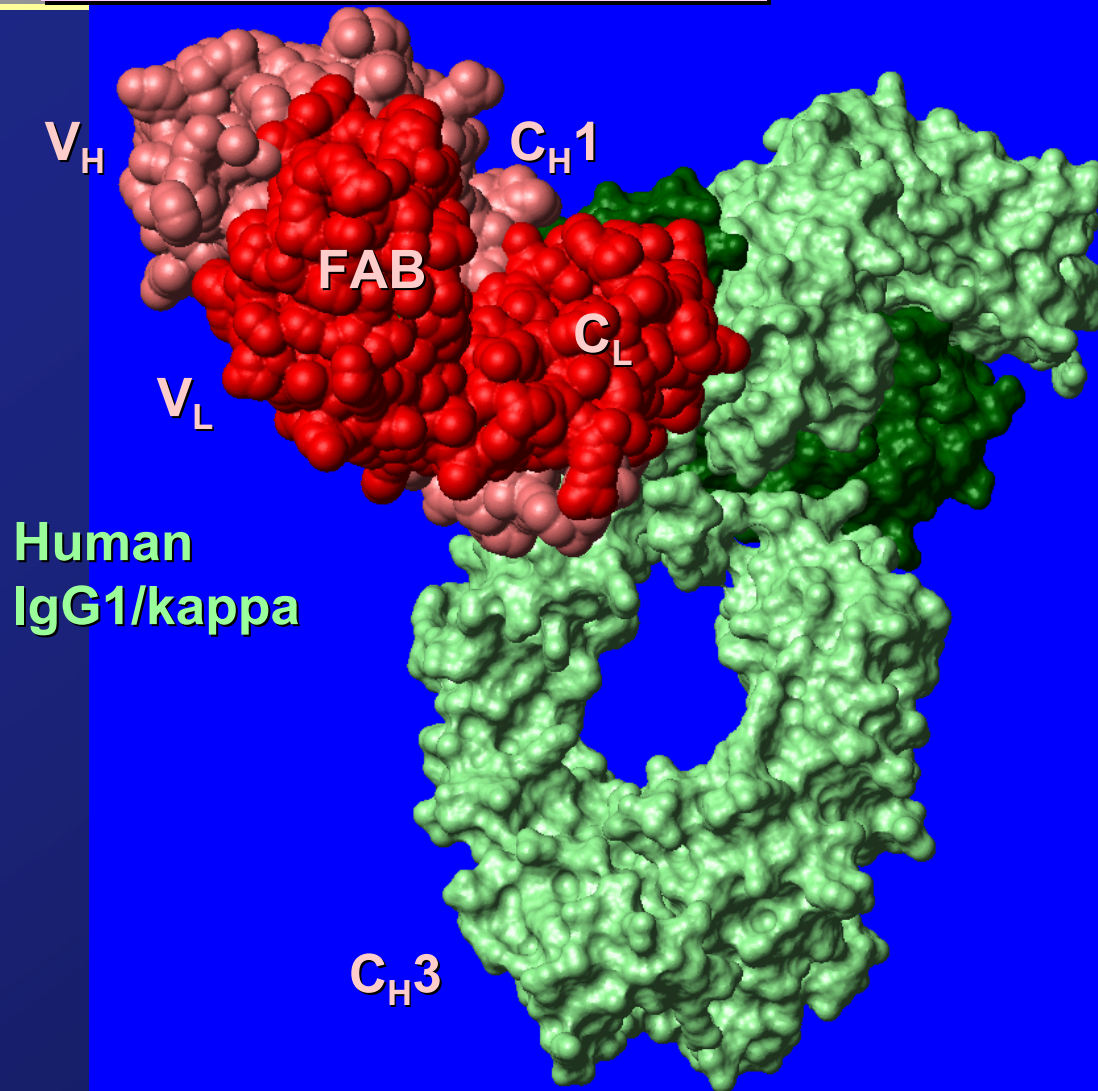
Thermal Unfolding Behavior of C_H3



Structural Consequences of Optimized Mutations



Beyond Consensus to Stabilize Poorly Behaved Antibody Domains

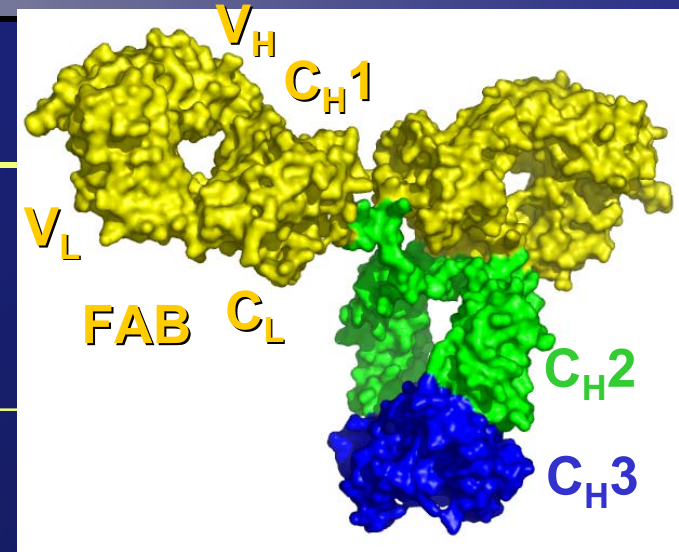
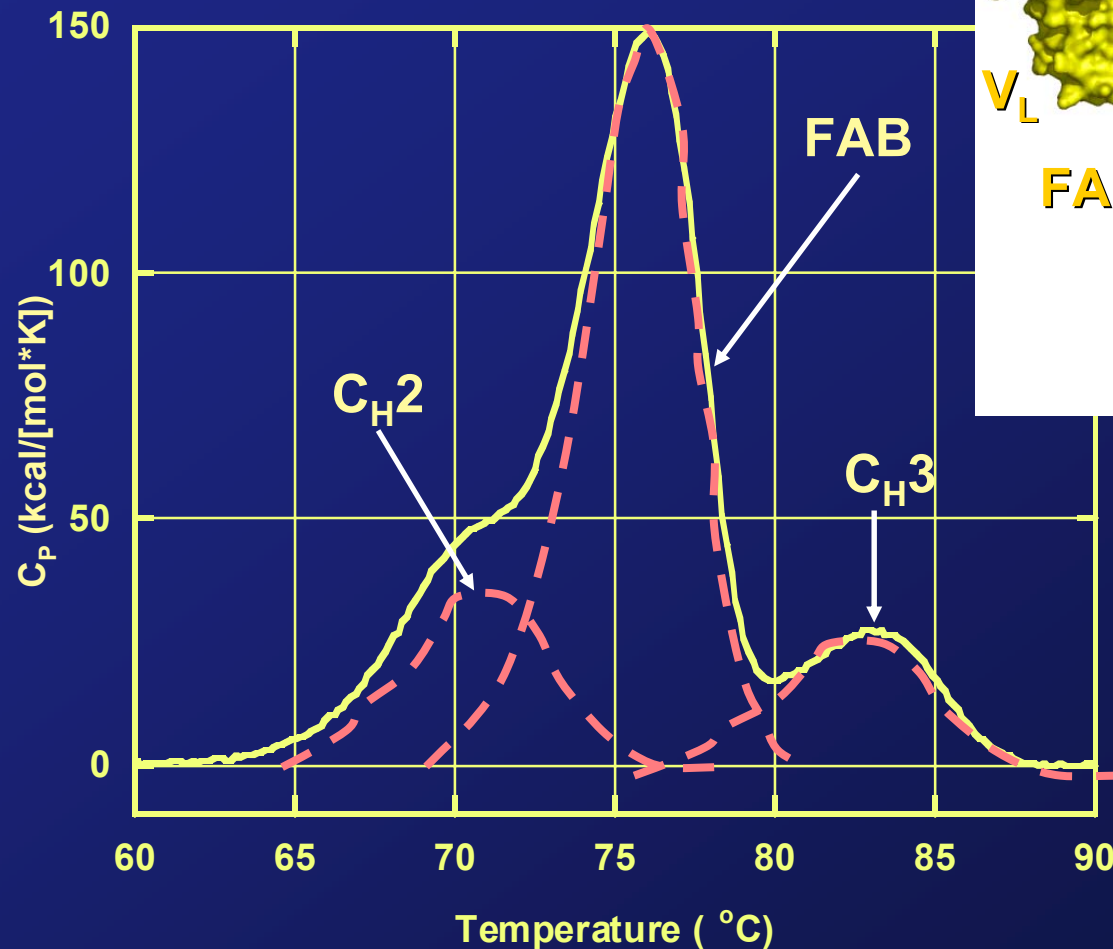


- Human FAB
- Bacterial expression level ~0.3 mg/L
- Variable function from prep to prep
- *Domain dependent stabilization
- *Functional/Folding consequences

General Protein Engineering Observations

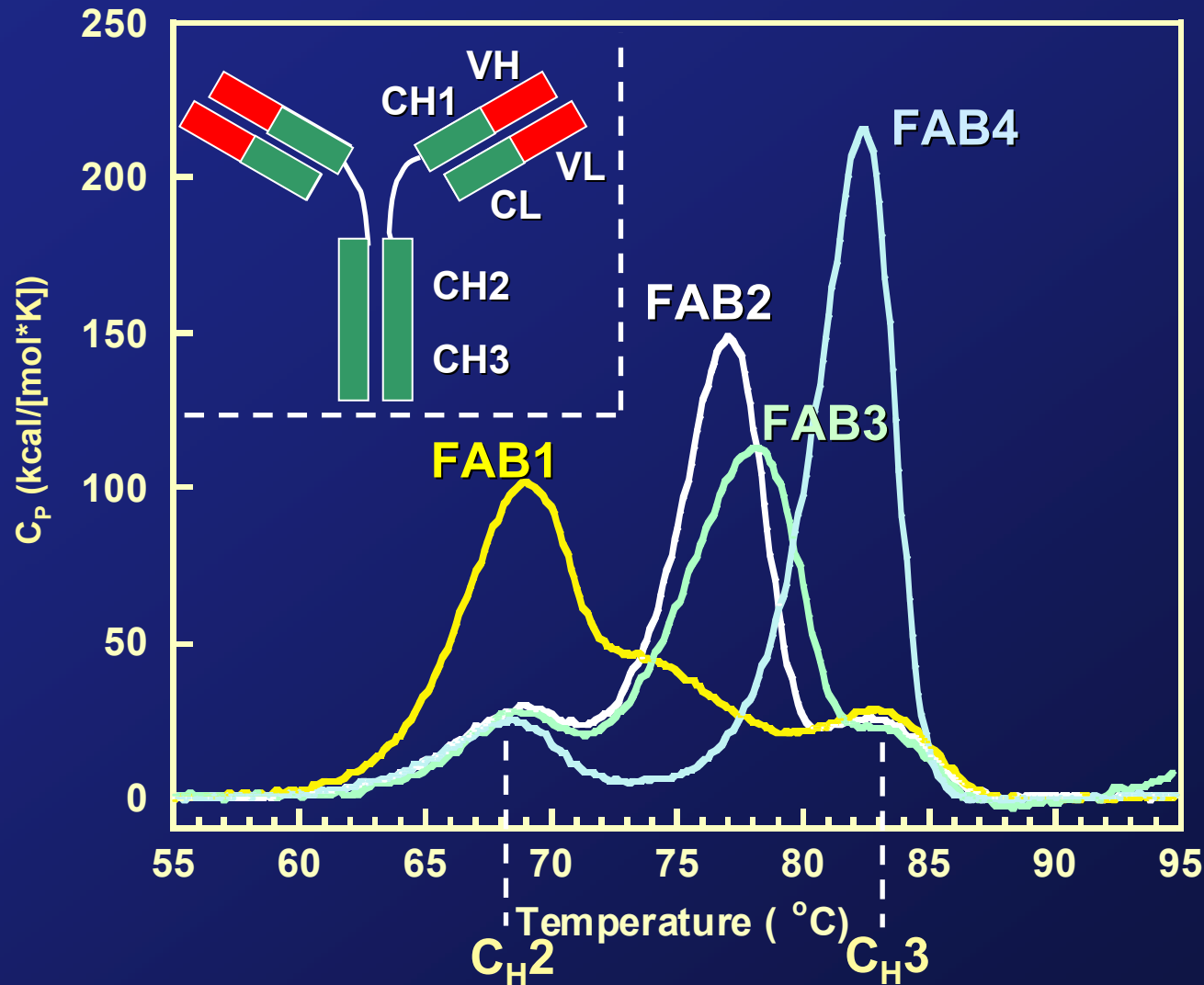
- **Stabilization can lead to increased expression.**
- **Stabilization can enhance product quality and functionality, especially in non-mammalian expression hosts.**
- **Adequate design of sequence databases provides reliable information regarding the tolerability of amino acids at various positions within antibody domains (protein domains).**

Human IgG1 Thermal Unfolding as a WHOLE



↑
Saphire et al., *Science*
(2001) V. 293.

Variation in Human IgG1 Stability Based on Variable Domains



IgG Sequence/Stability Correlations

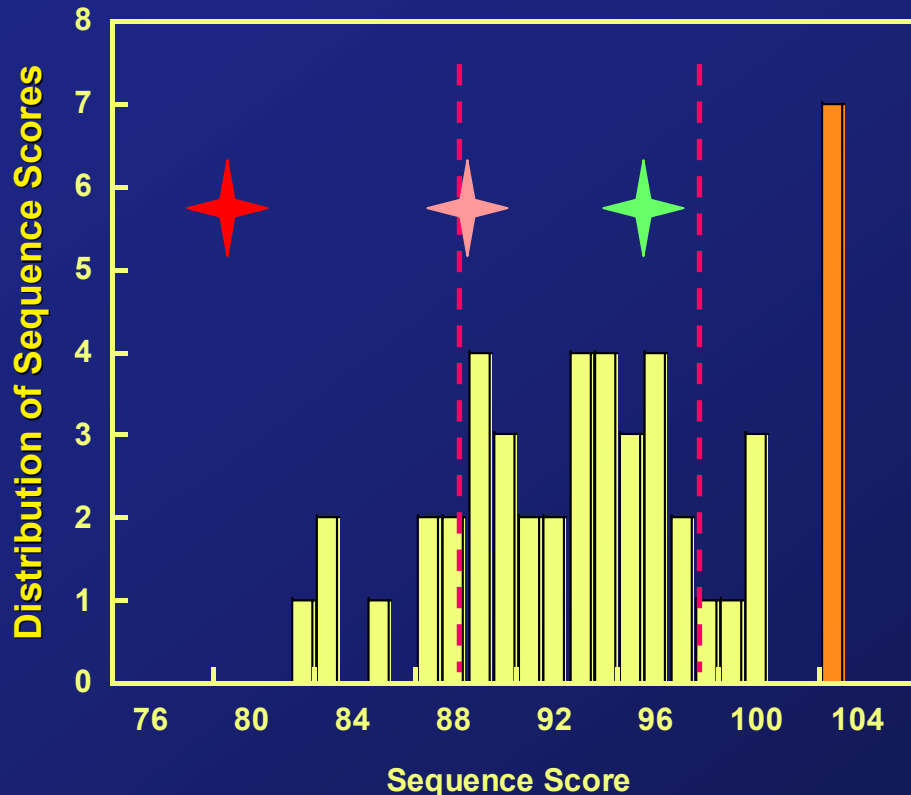
Can antibody VARIABLE DOMAIN sequences be used to predict Antibody Stability/Expression?

We trust the mammalian antibody databases for stability engineering, but can we use the sequence information for *de novo stability prediction*?

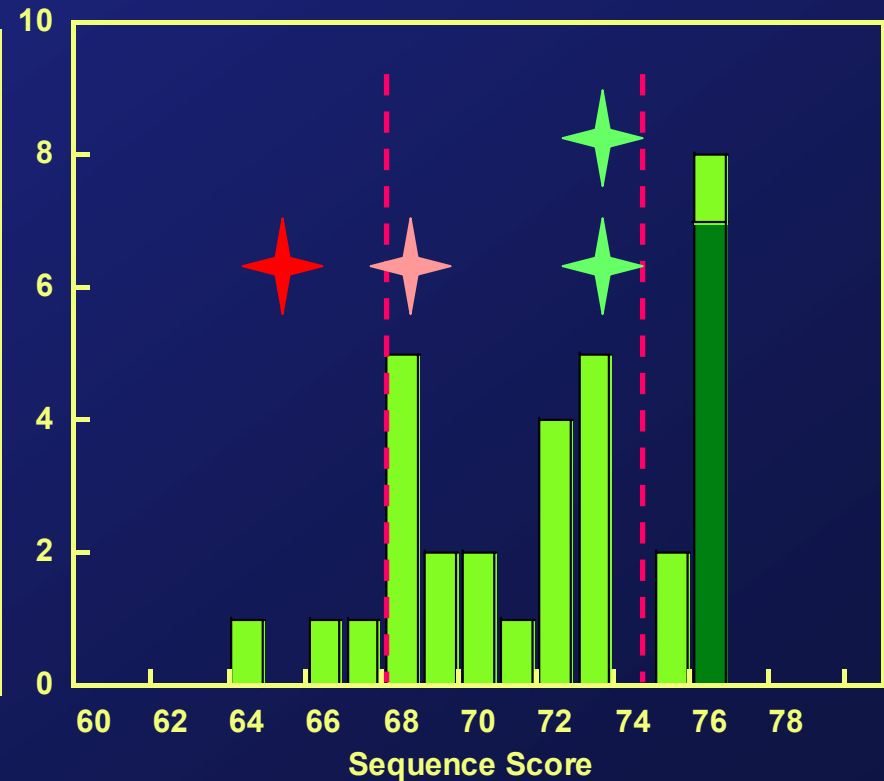
- Developed a Scoring Algorithm Which Compares individual Variable Domain Sequence of Interest to the entire Database of Mammalian Sequences

How Do Natural Sequences Which Score Badly Behave *In Vitro*?

VH3 Subclass



VH1 Subclass

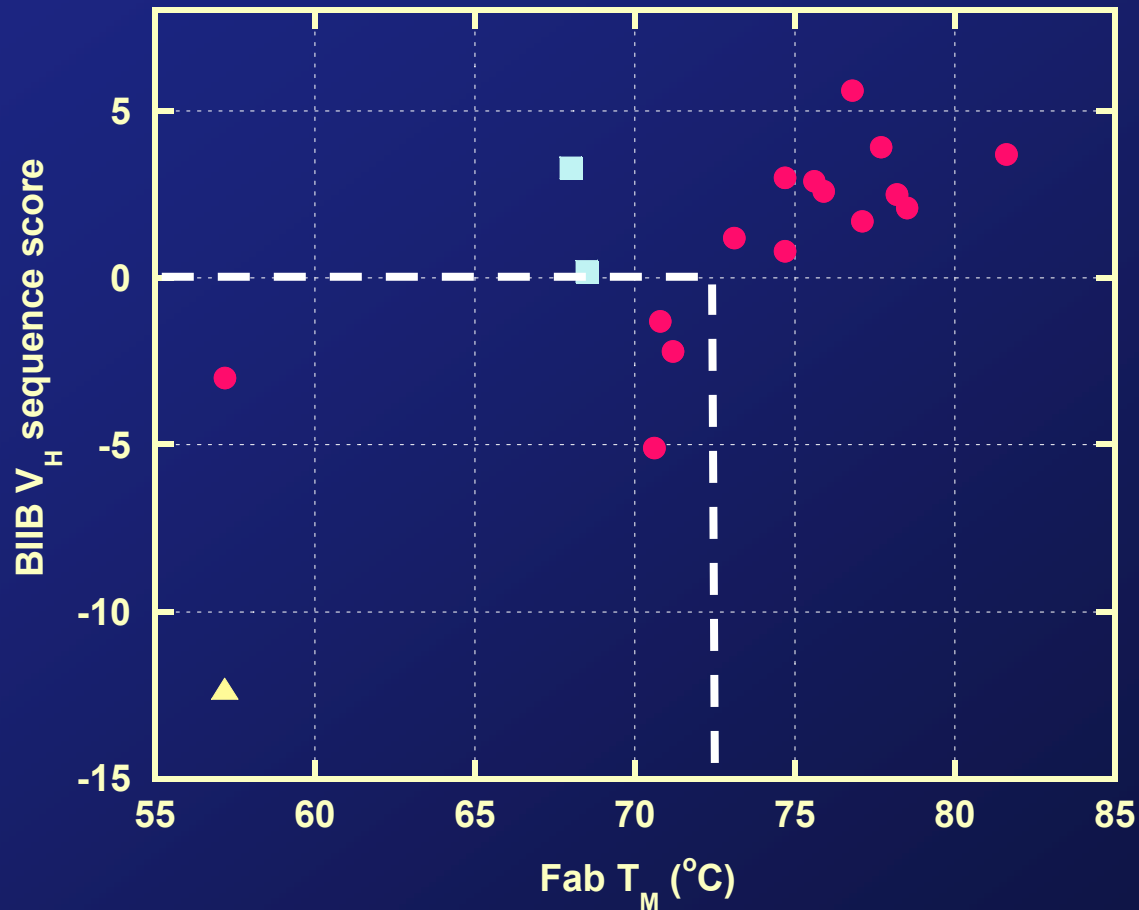


~20 Biogen Idec Preclinical or Clinical Phase Human(ized) Antibodies (1) **Scored** AND (2) **FAB Stability Tested**.

18 BIIB Human(ized) V_H Scores Correlate Roughly with Stability

IgG	Fab T _M (°C)	V _H sub- class	V _H germline ^a	V _H score	V _H sub class score	Δscore	V _κ sub- class	V _κ germline ^a	V _κ score	V _κ subclass score	Δscore
BIIB1	81.6	VH1	X92340VH1	73.8	70.1+/-3.1	+3.7	VK1	M64855VK1	78.2	80.3+/-3.5	-2.1
BIIB2	78.5	VH1	X92340VH1	72.2	70.1+/-3.1	+2.1	VK1	M64855VK1	72.8	80.3+/-3.5	-8.5
BIIB3	78.2	VH1	AB019438VH1	72.6	70.1+/-3.1	+2.5	VK2	X12684VK2	82.4	85.3+/-2.1	-2.9
BIIB4	77.7	VH3	M99649VH3	96.1	92.2+/-4.5	+3.9	VK2	X12684VK2	83.9	85.3+/-2.1	-1.4
BIIB5	77.1	VH2	X56365VH4	69.8	68.1+/-5.5	+1.7	VK1	X59316VK1	83.0	80.3+/-3.5	2.7
BIIB6	76.8	VH3	M99649VH3	97.7	92.2+/-4.5	+5.6	VK1	M64855VK1	77.4	80.3+/-3.5	-2.9
BIIB7	75.9	VH1	AB019438VH1	72.7	70.1+/-3.1	+2.6	VK3	X72812VK3	83.2	84.1+/-2.4	-0.9
BIIB8	75.6	VH1	Z14300VH1	73.0	70.1+/-3.1	+2.9	VK1	X59316VK1	79.8	80.3+/-3.5	-0.5
BIIB9	74.7	VH3	Z12358VH3	95.2	92.2+/-4.5	+3.0	VK4	Z00023VK4	88.8	92.6+/-2.6	-3.8
BIIB10	74.7	VH4	X56365VH4	69.3	68.1+/-5.5	+0.8	VK1	X59316VK1	84.0	80.3+/-3.5	3.7
BIIB11	73.1	VH1	AB019438VH1	71.3	70.1+/-3.1	+1.2	VK4	Z00023VK4	94.4	92.6+/-2.6	1.8
BIIB12	71.2	VH1	AB019438VH1	67.9	70.1+/-3.1	-2.2	VK4	Z00023VK4	92.5	92.6+/-2.6	-0.1
BIIB13	70.8	VH3	M99657VH3	91.1	92.2+/-4.5	-1.3	VK4	Z00023VK4	93.1	92.6+/-2.6	0.5
BIIB14	70.6	VH7	L10057VH7	65.1	70.2+/-3.2	-5.1	VK-	-	56.6	-	-
BIIB15	68.5	VH3	M99660VH3	92.4	92.2+/-4.5	+0.2^b	VK1	M64858VK1	81.6	80.3+/-3.5	1.3
BIIB16	68.0	VH3	M99649VH3	95.6	92.2+/-4.5	+3.3^b	VK3	X72812VK3	82.0	84.1+/-2.4	-2.1
BIIB17	57.2	VH3	J00239VH3	89.4	92.2+/-4.5	-3.0	VK2	X63397VK2	84.8	85.3+/-2.1	-0.5
BIIB18	- ^c	VH3	M99400VH3	80.0	92.2+/-4.5	-12.3^b	VK2	X63397VK2	84.5	85.3+/-2.1	-0.8

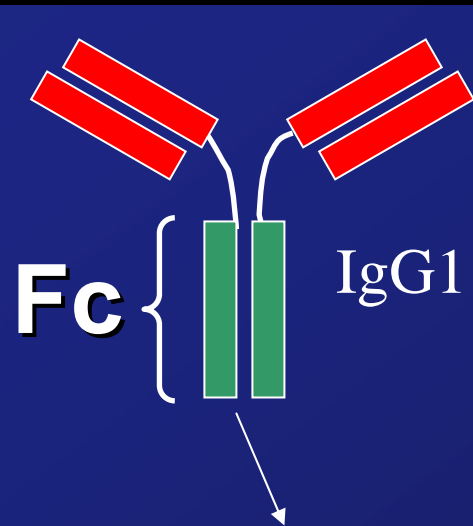
Low Sequence Scores Trend With Low Stability Antibodies



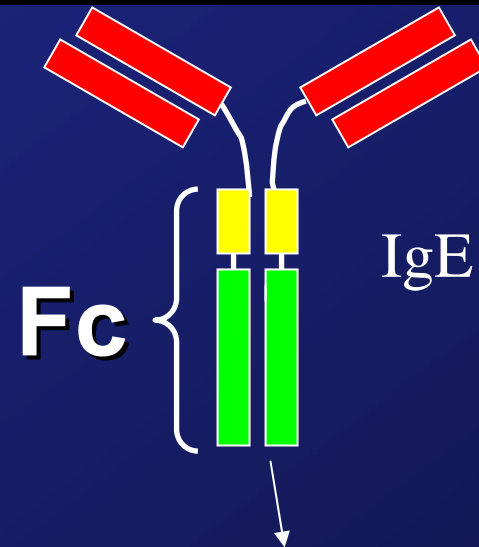
SUMMARY

- Low V_H Sequence Scores *TREND* towards lower stability antibodies. No trend observed with V_L sequences.
- Scoring can (should) be used as an initial tool to identify potential problems with therapeutic antibodies or with antibodies isolated from library approaches.
- Scores do not give the entire story –
 - (i) certain residue positions more important for stability than others
 - (ii) V-(D-)J-C joining can affect stability
 - (iii) V_H/V_L Compatibility not accounted for

Structural Aspects of IgE versus IgG



IgG1 Fc ($Fc\gamma$)

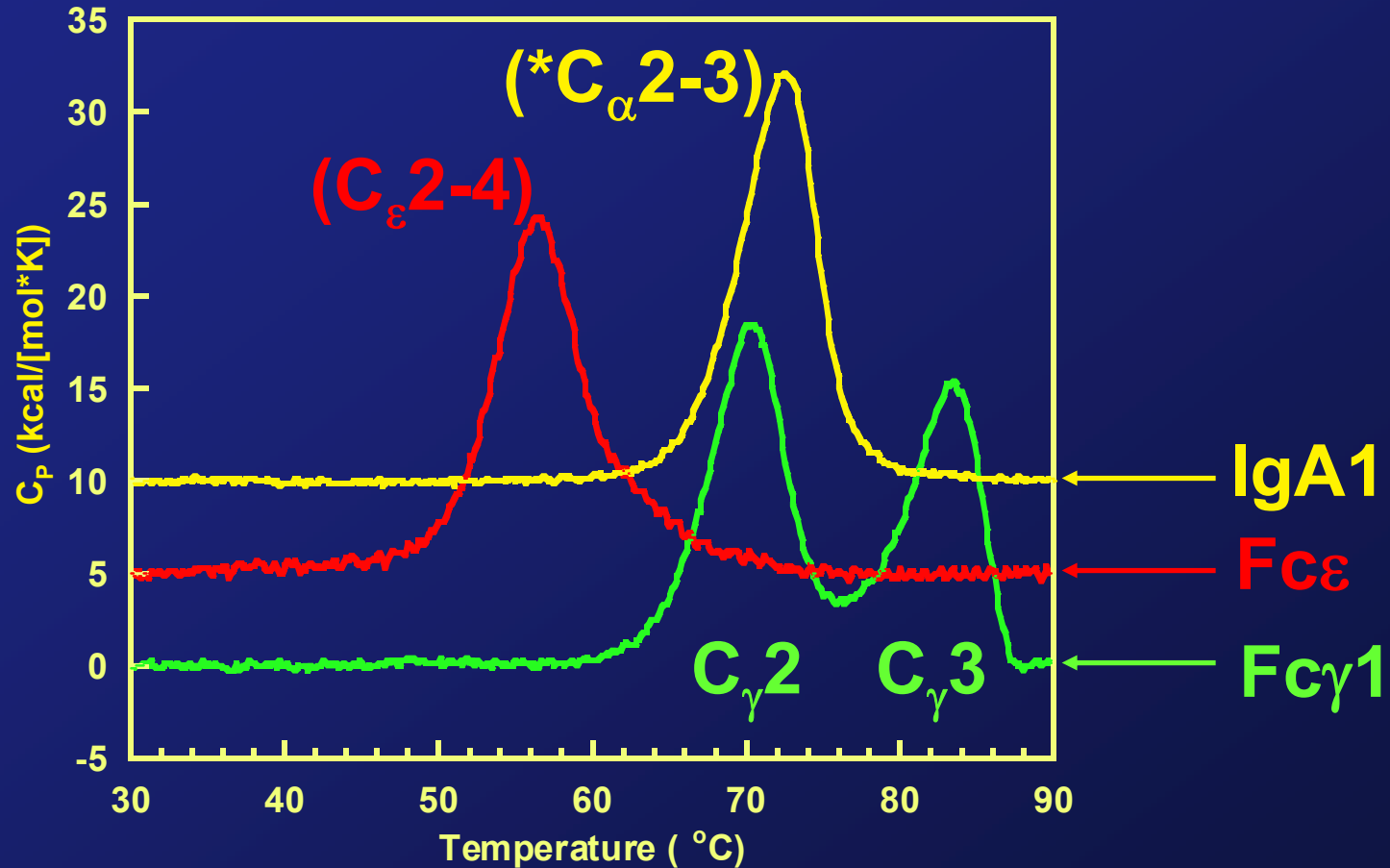


IgE Fc ($Fc\epsilon$)

See labs of Gould, Jardetzky and Cowburn...

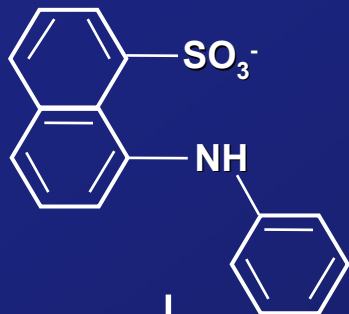
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Antibody Isotype Fc Stability

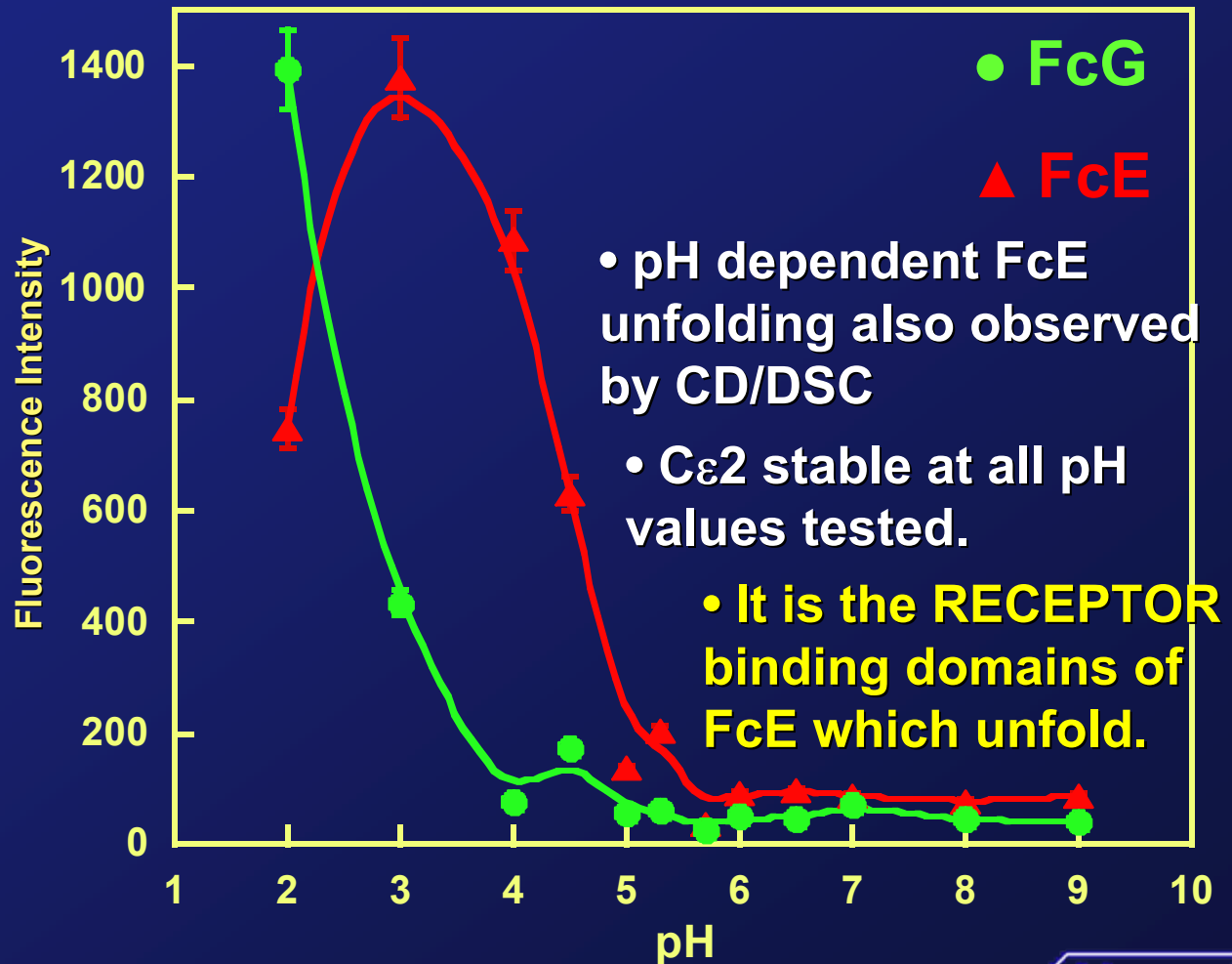


Conformational Changes Evident in FcE at Intermediate pH Levels

ANS



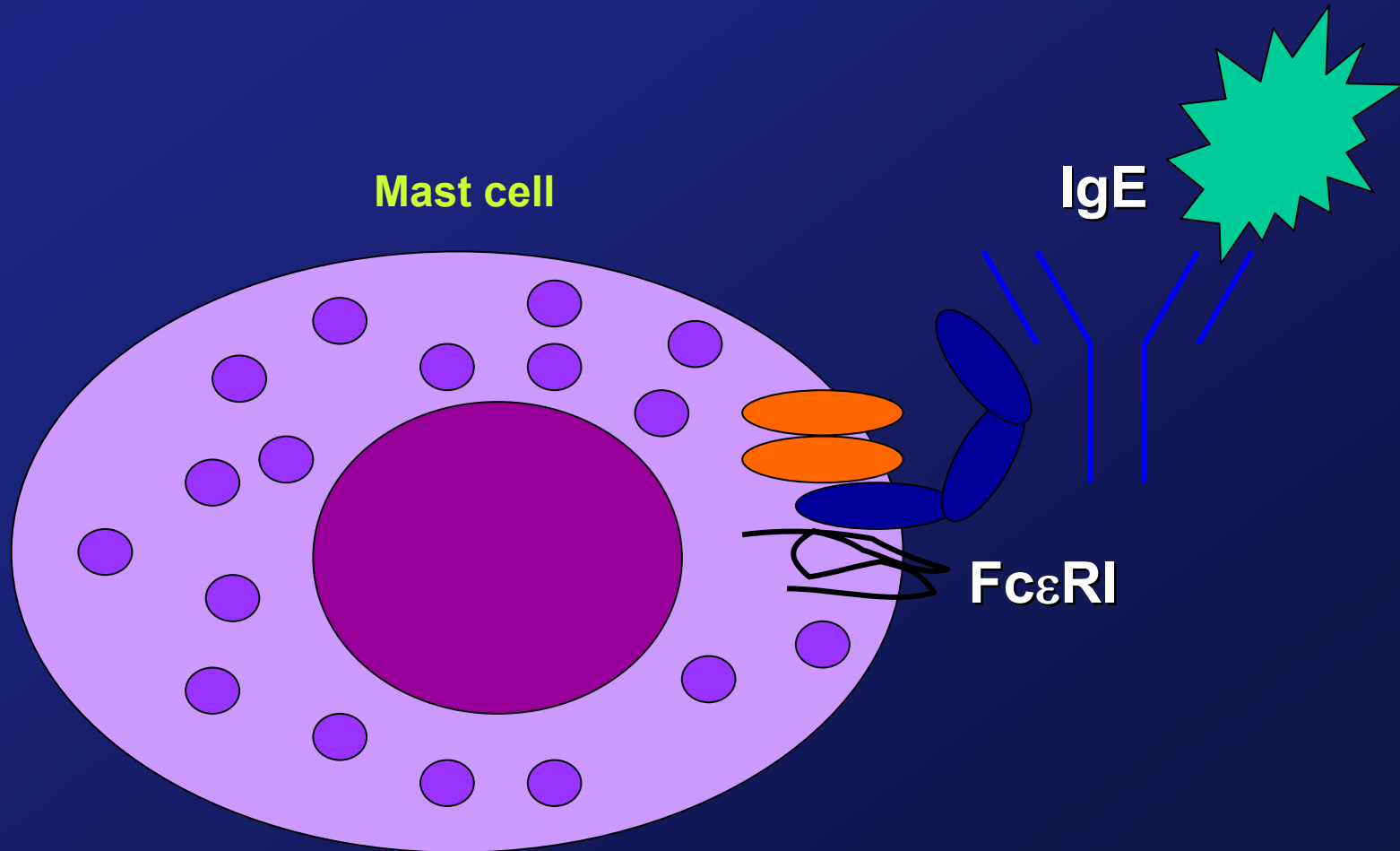
$N \rightleftharpoons MG \rightleftharpoons U$



Functional Properties of the IgE Fc

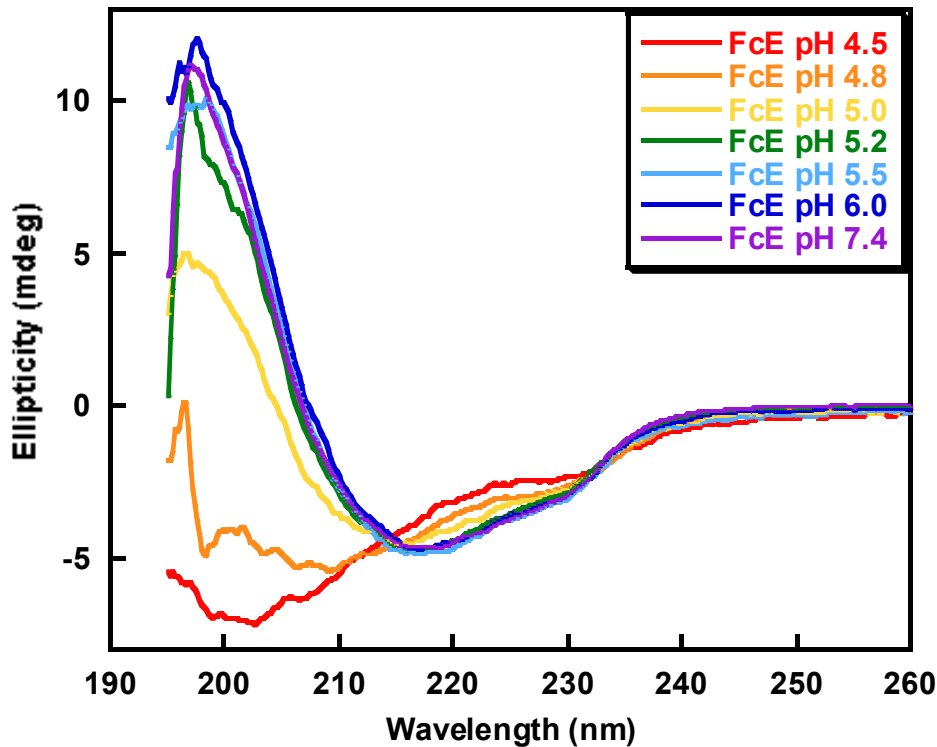
- **FcERI mediated signaling in allergy and inflammation**
- **CD23 mediated signaling/transport**

IgE/FcERI Mediated Degranulation

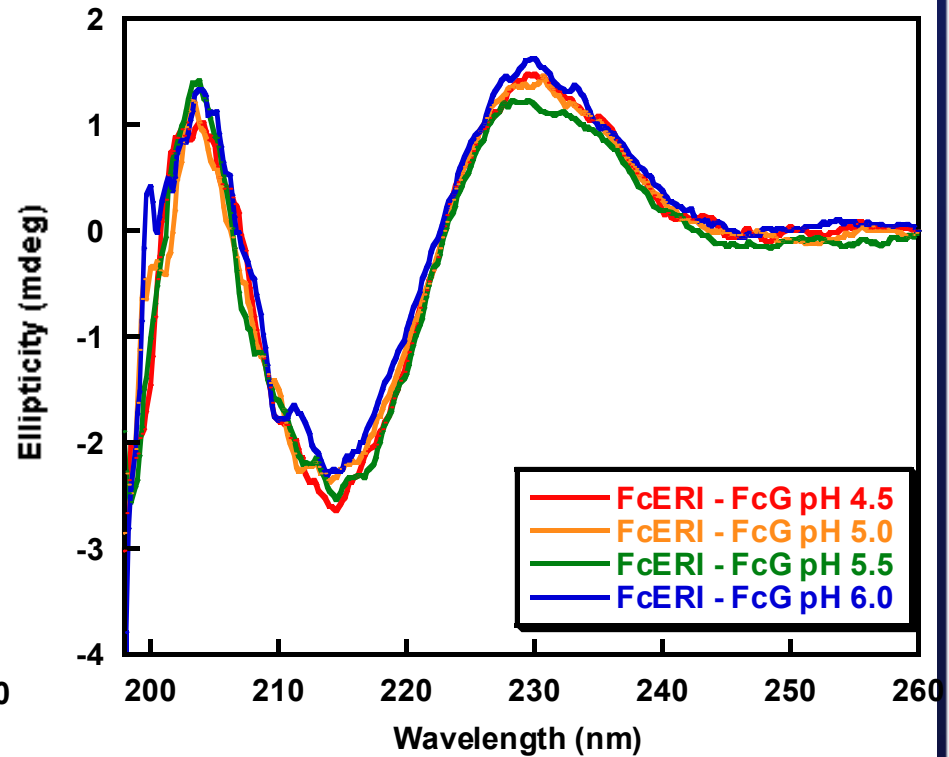


FcERI α is stable at pH values where FcE unravels

FcE



sFcERI α



Interaction Between FcE and FcERI Probed by DSC

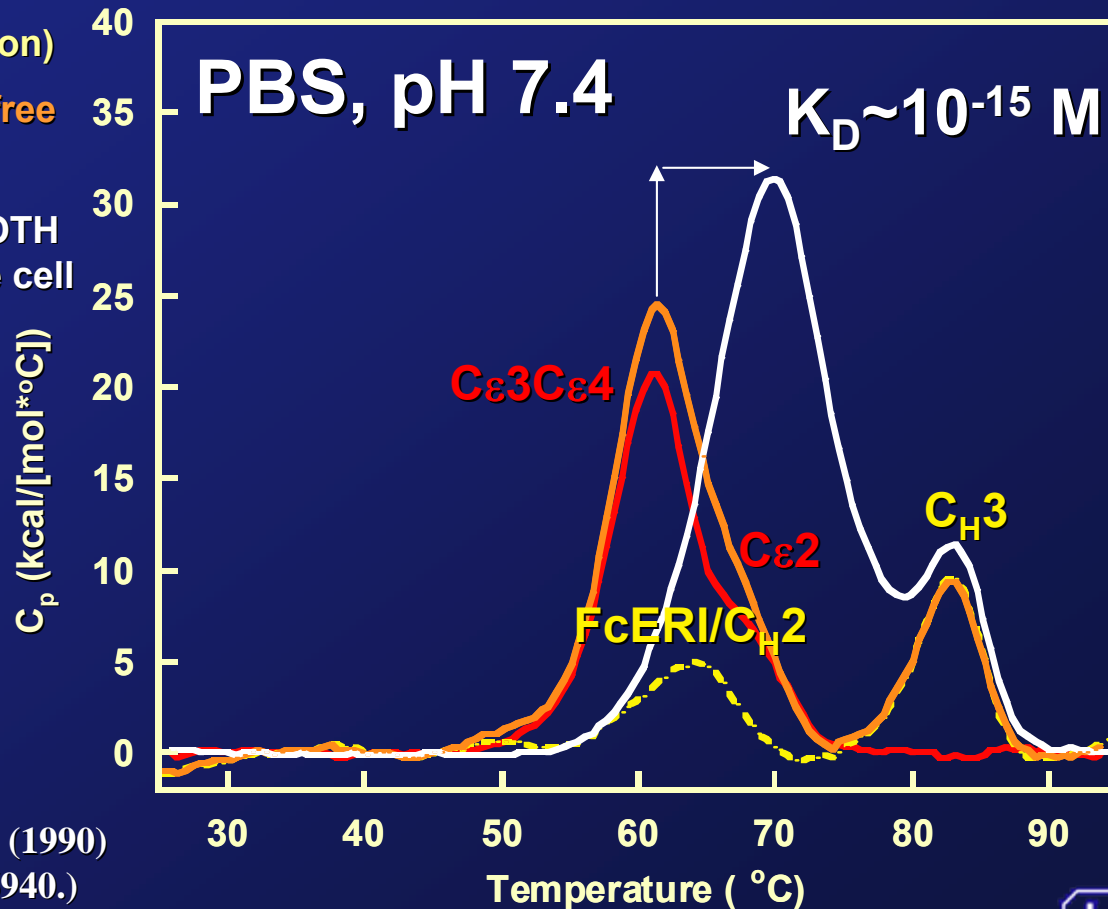
$K_D < 10^{-10-11}$ (M) SPR*

Red = FcE (C ϵ 2C ϵ 3C ϵ 4)

Yellow = FcG-Fc ϵ R1 α (fusion)

Orange = Addition of the free proteins' DSC curves

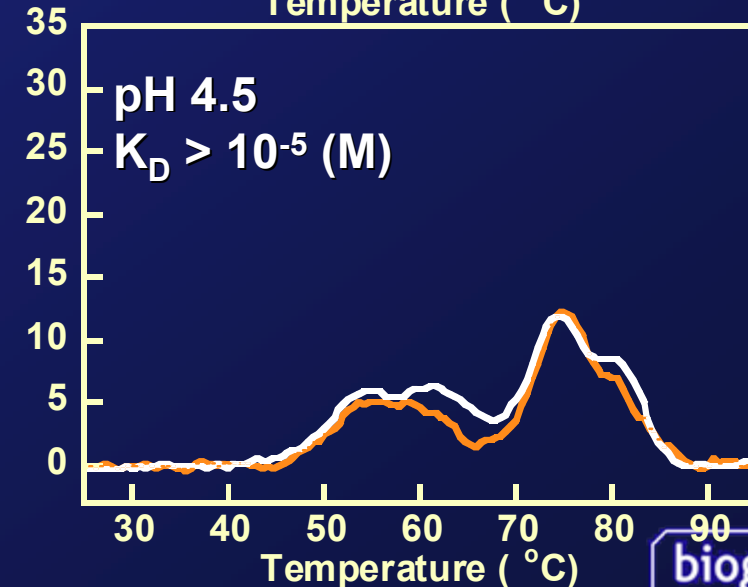
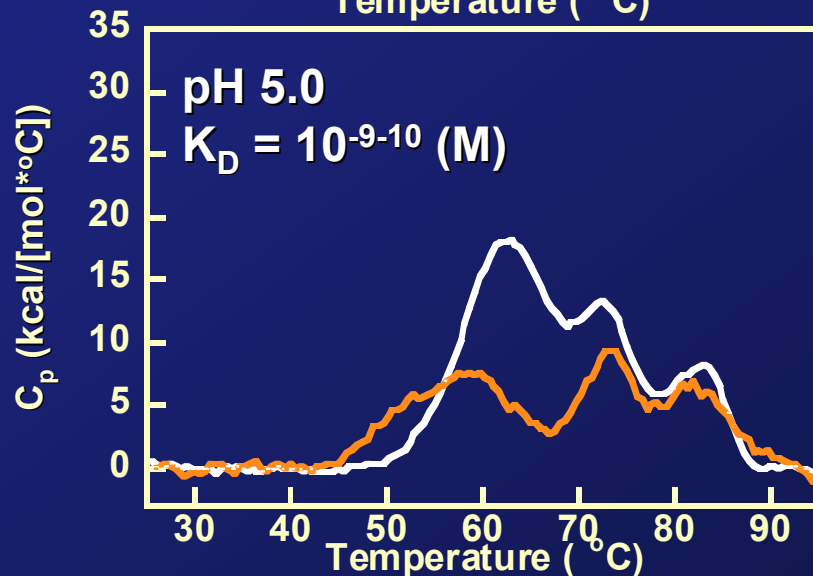
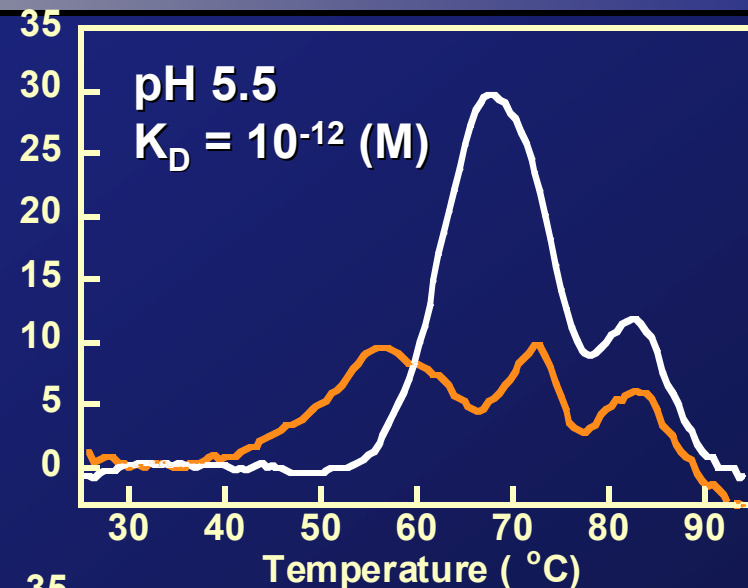
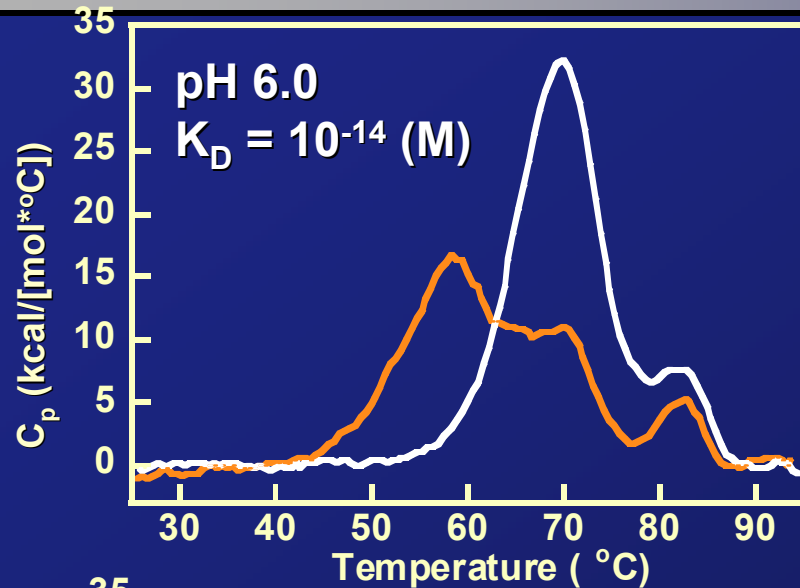
White = DSC scan with BOTH Fc ϵ and FcG-Fc ϵ R1 α in the cell



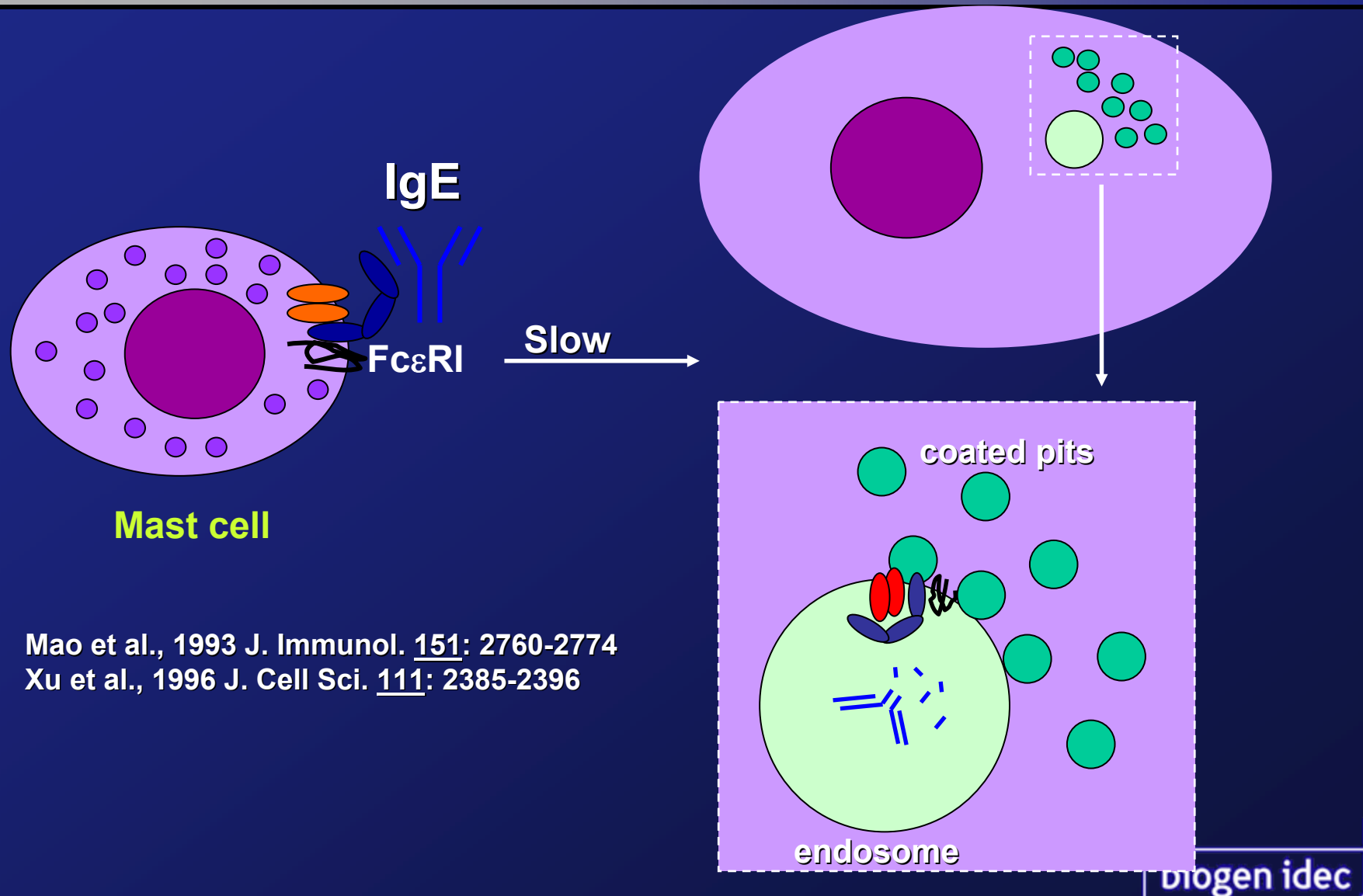
(Brandts, J.M and Lin L.-N. (1990)
Biochemistry. 29, pp. 6927-6940.)

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pH Dependence of the FcE/FcERI Interaction



How is IgE Naturally Degraded?



Summary of IgE-Fc Characterization

- **FcE is intrinsically much less stable than other Ab isotypes.**
- **pH dependent unfolding of FcE at pH 5 abrogates its interaction with FcERI.**
- **pH dependent unfolding may provide an novel mechanism for IgE regulation.**

Acknowledgements

Biogen Idec

Department of Analytical and
Protein Chemistry

FcE

Jennifer Hopp, Julie Chung, Mike
Shields, Mike LaBarre, Karen
Hathaway, Carmen Young, Flora
Huang, Dustin Lloyd, XianJun
Cao, Marilyn Kehry, Konrad
Miatkowski, Werner Meier

Stability Predictions

Fred Taylor, Flora Huang, Arlene
Sereno, Julia Coronella, Robert
Peach, LiYing Jiang, Scott
Glaser

Syngenta

TMRI

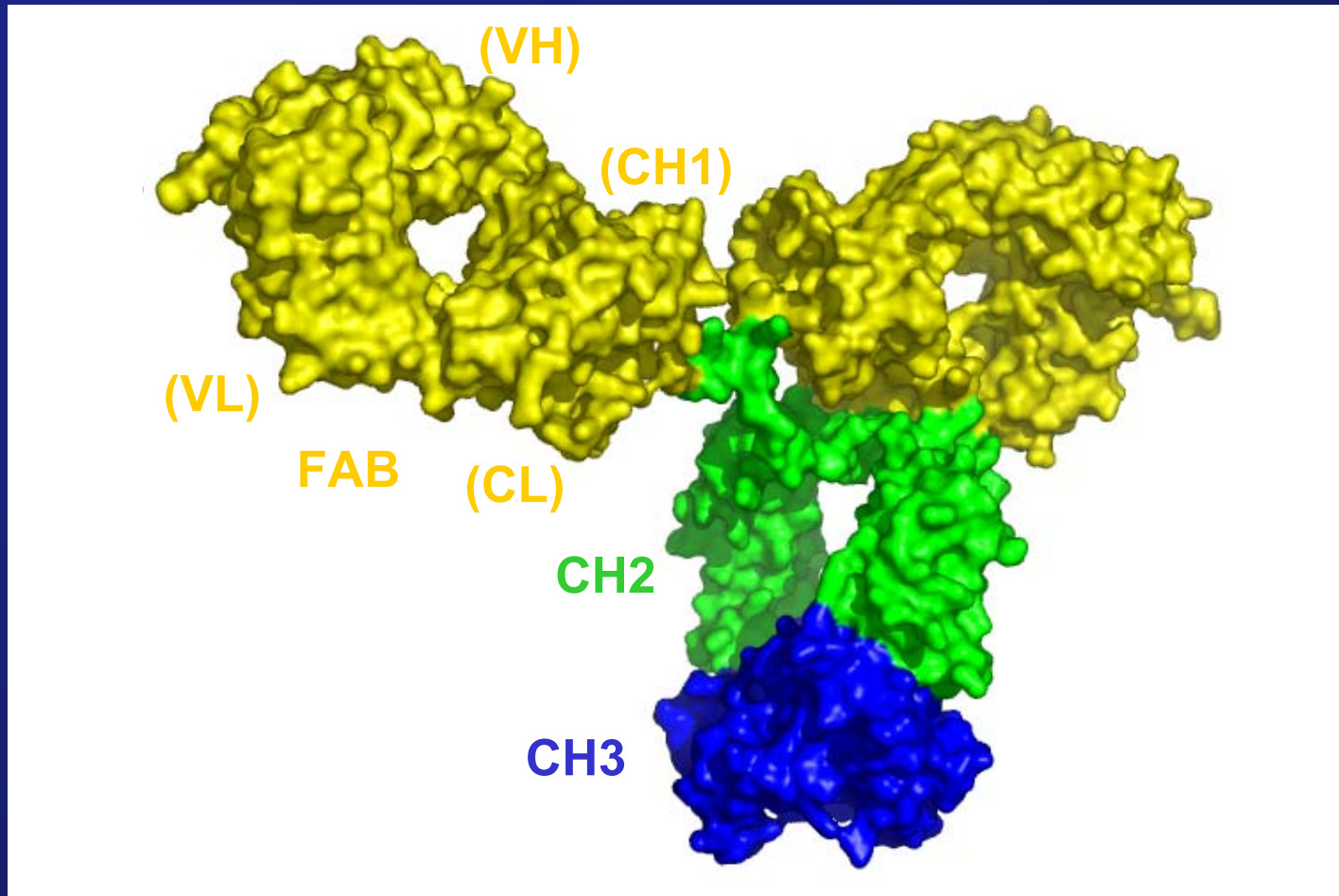
Genevieve Hansen, Jeff Rogers

MicroCal

Verna Frasca, Dile Holton, Eric
Reese, Steve Spotts, William
Gelb, Brett Treganowan

BACK-UPS

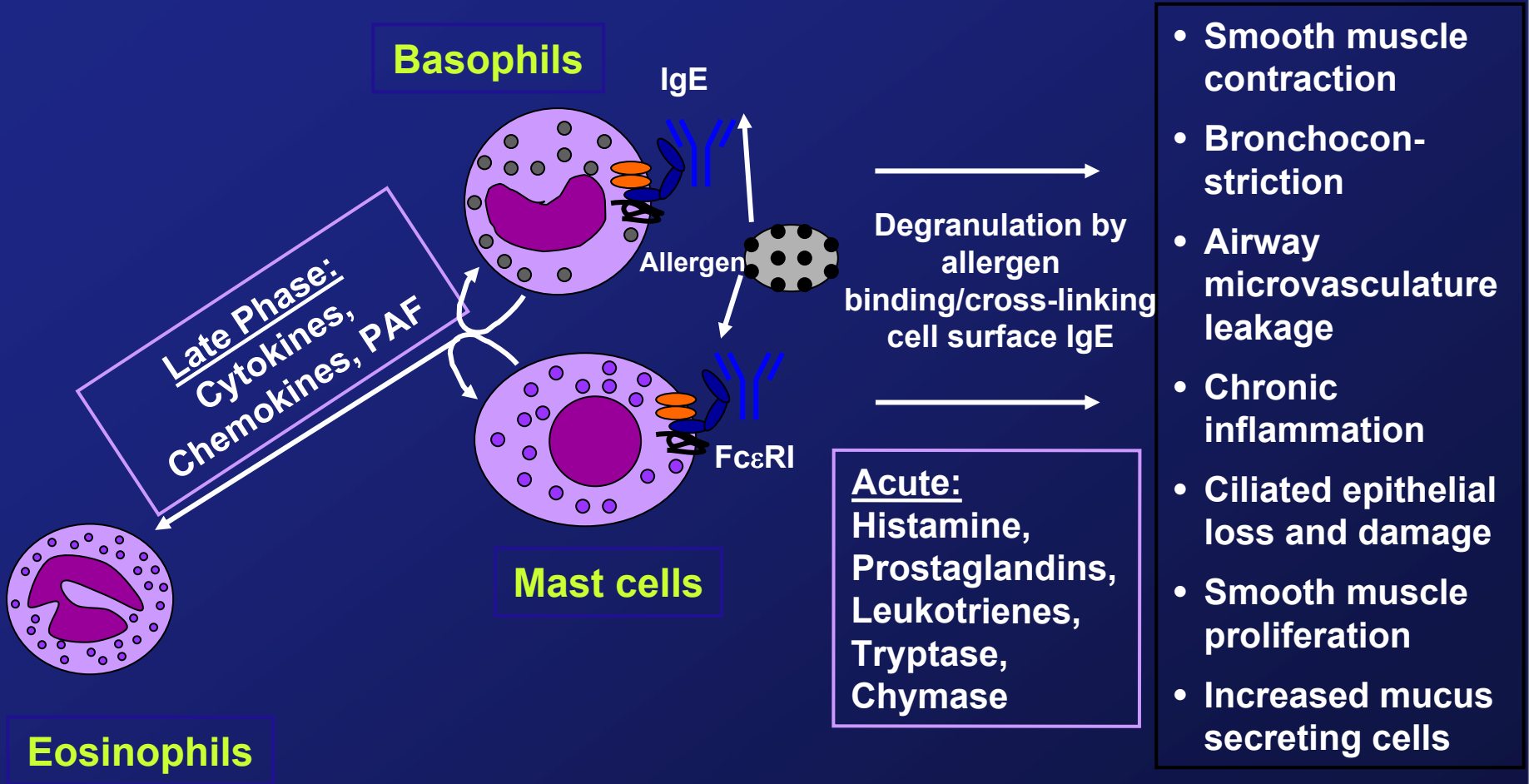
IgG Structure in Terms of Cooperatively Folded Subunits

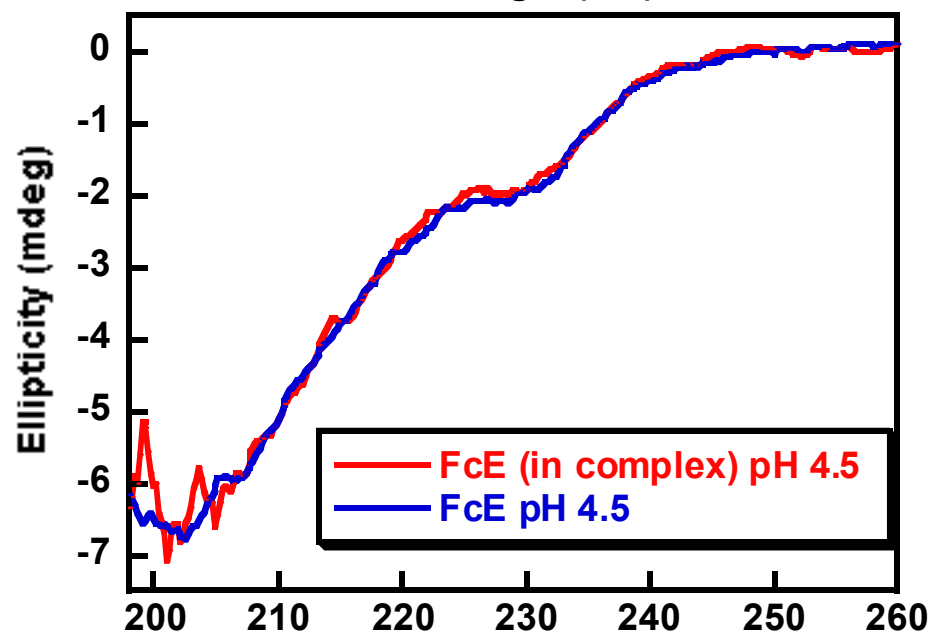
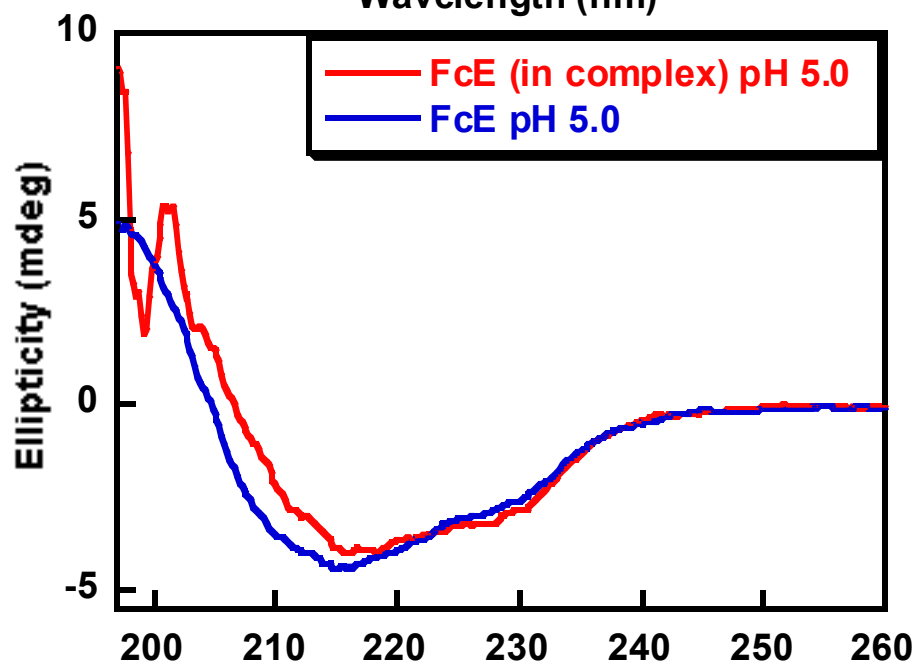
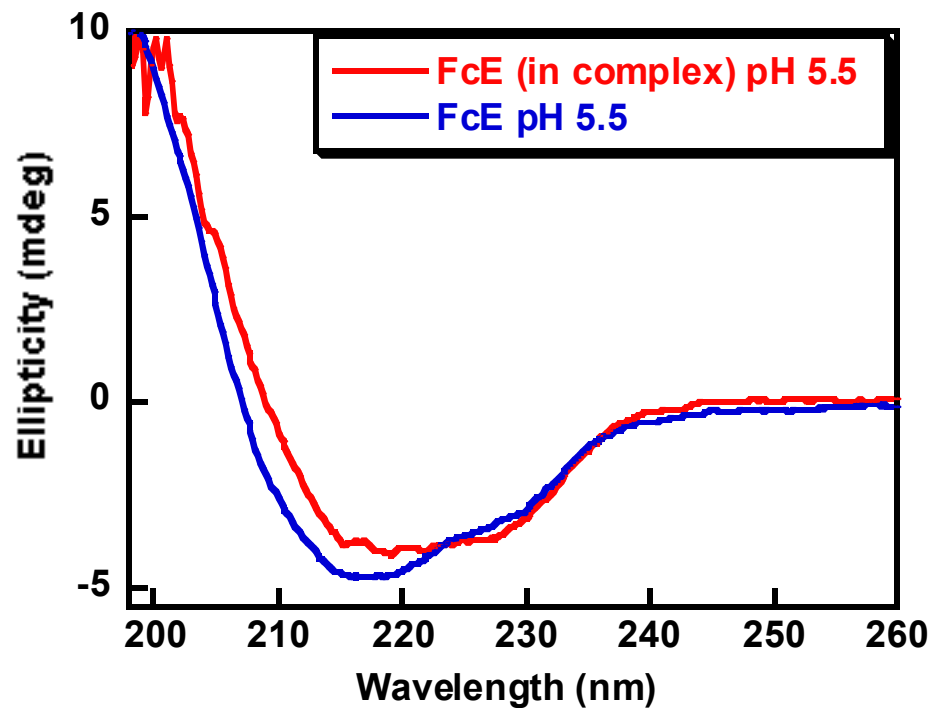
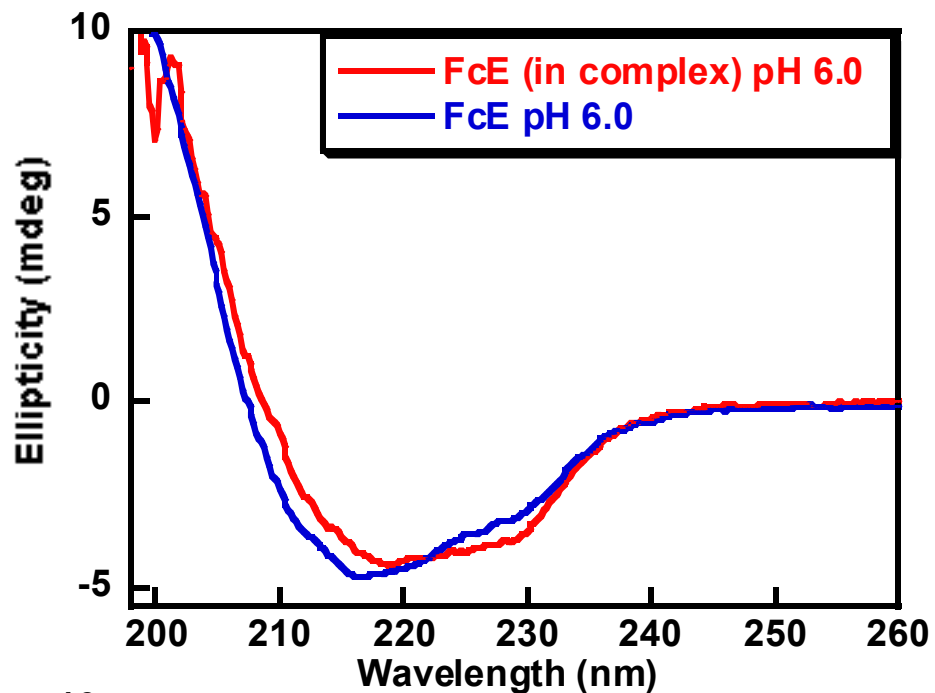


Saphire et al., 2001 Science, 293

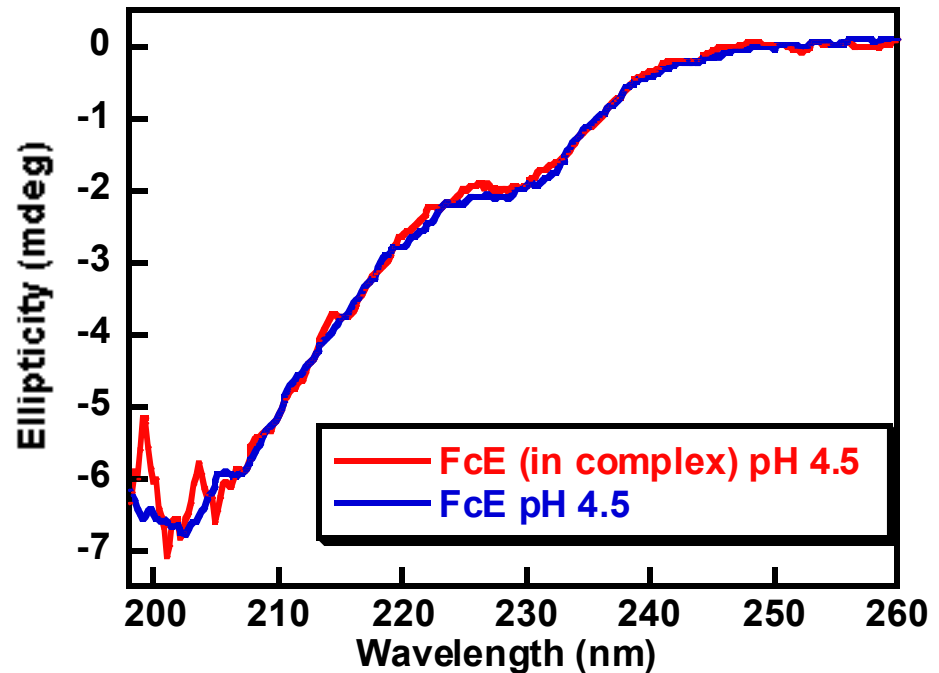
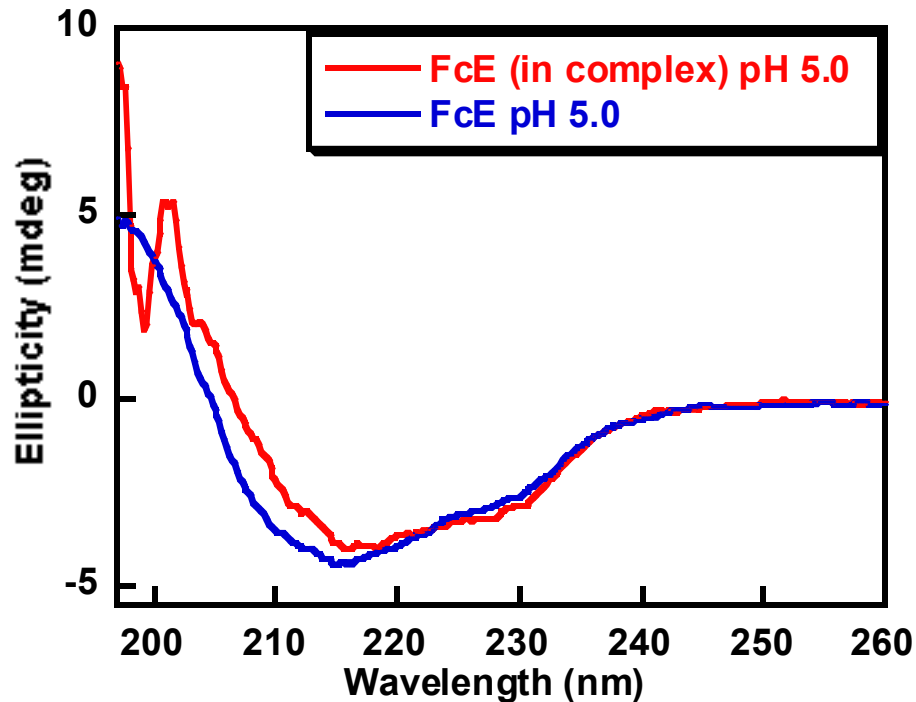
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IgE Mechanism of Action

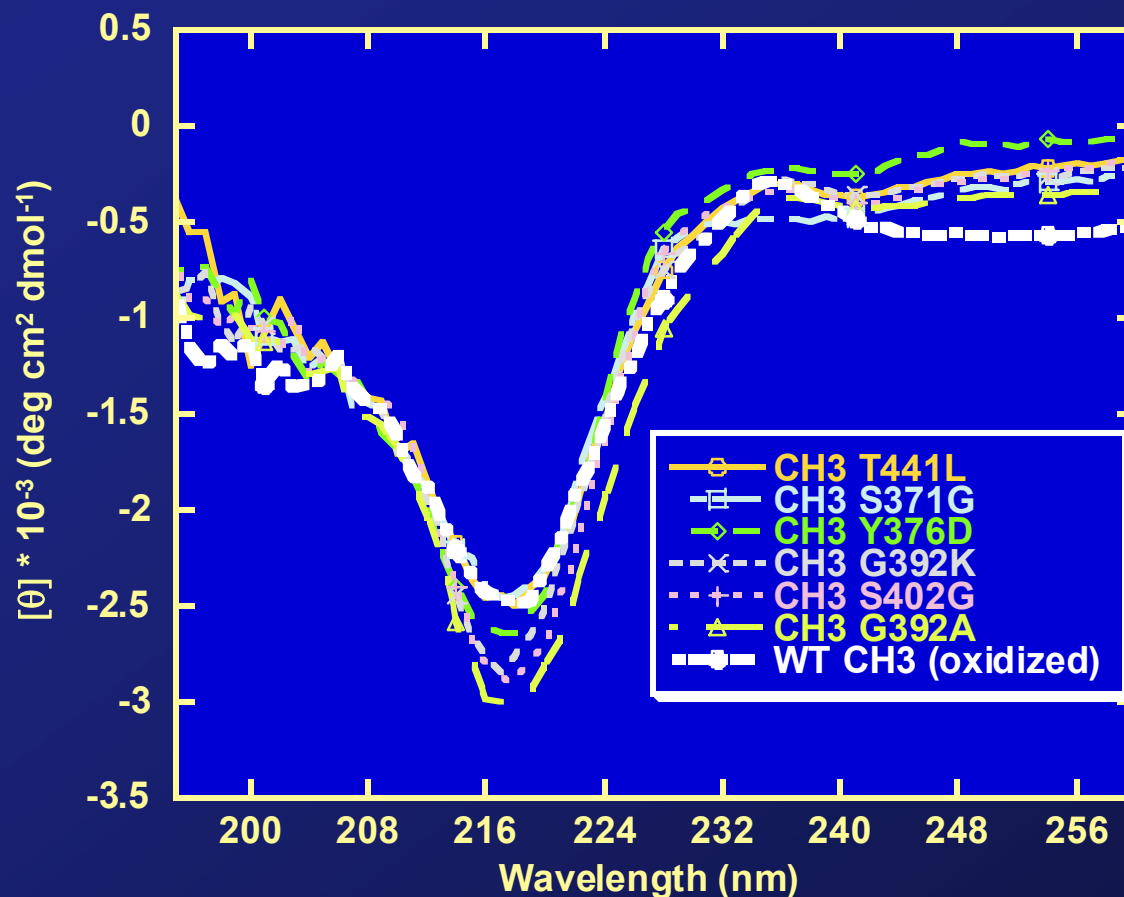




Loss of Interaction between FcE and FcERI α Correlates with FcE Unfolding



Structural Features of Mutant C_H3 Unchanged



All Domains Form Stable
Dimers

(even at concentrations
as low as 50 nM)

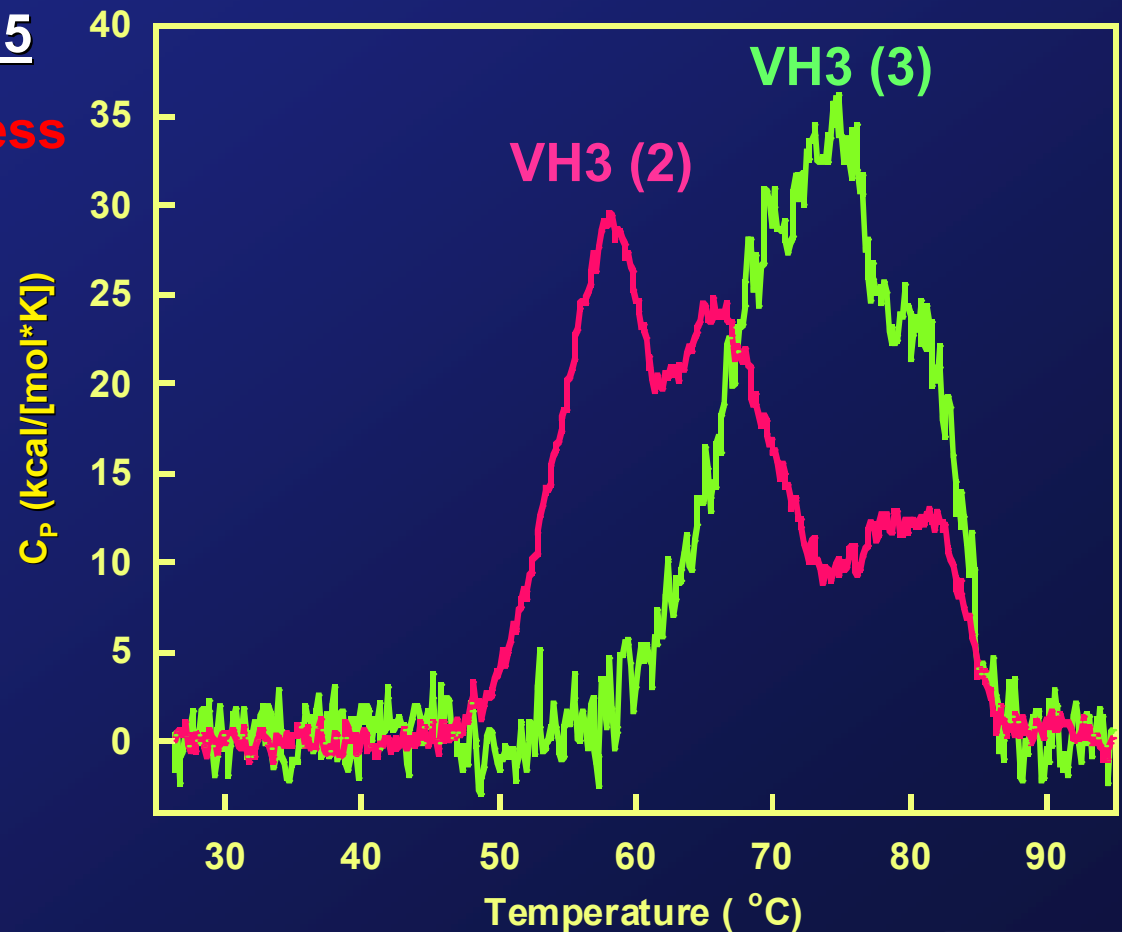
Stability Measurement of Human IgG1s with Different VH Scores

VH3 Avg Score = 93 ± 5

VH3 (1) = 79, No Express

VH3 (2) = 88

VH3 (3) = 95



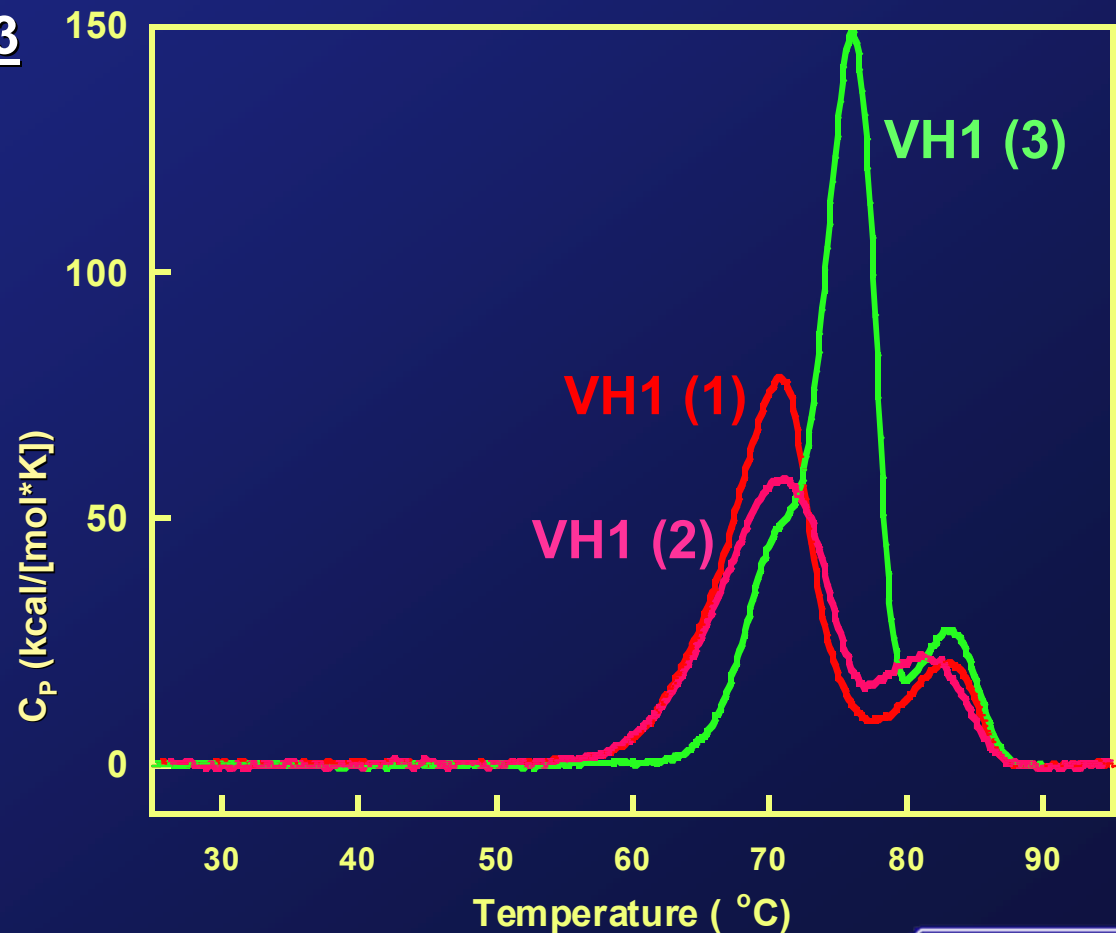
Stability Measurement of Human IgG1s with Different VH1 Scores

VH1 Avg Score = 71 ± 3

VH1 (1) = 65

VH1 (2) = 68

VH1 (3) = 73



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FcG is Natively Folded Above pH 3

pH 7

pH 6

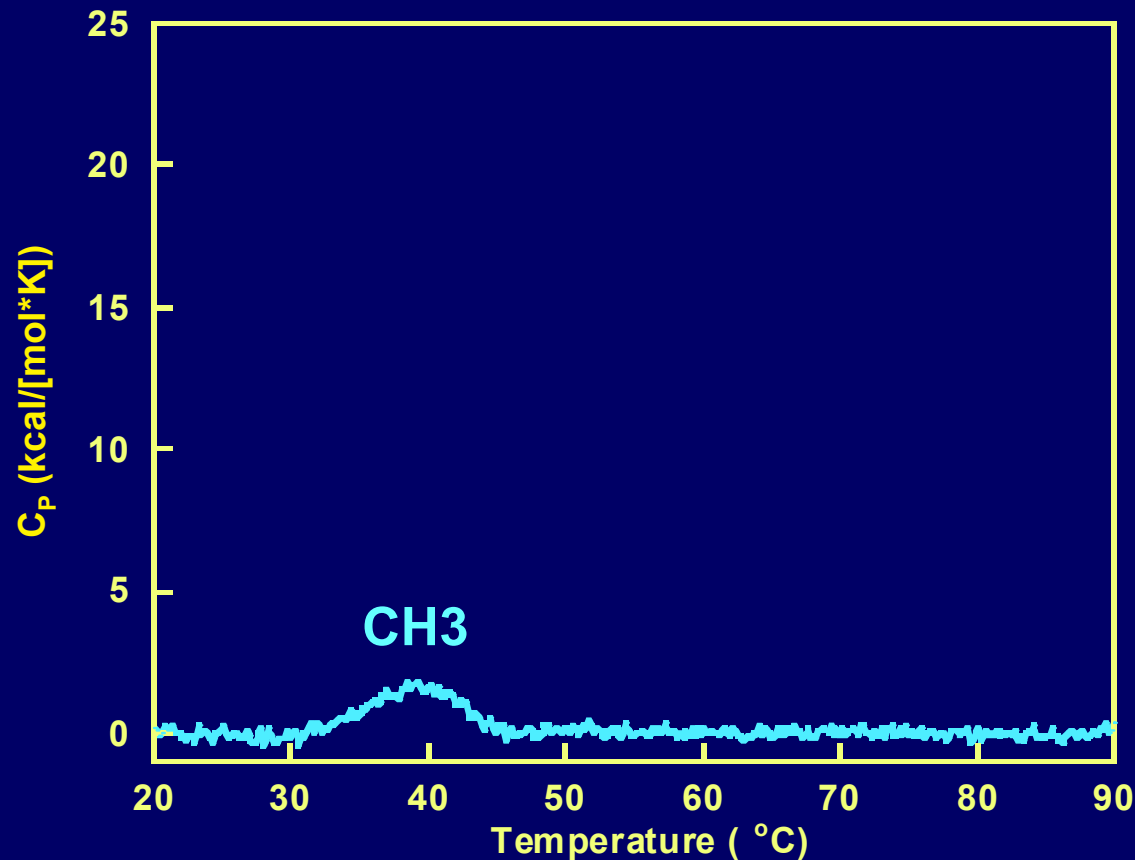
pH 5

pH 4.5

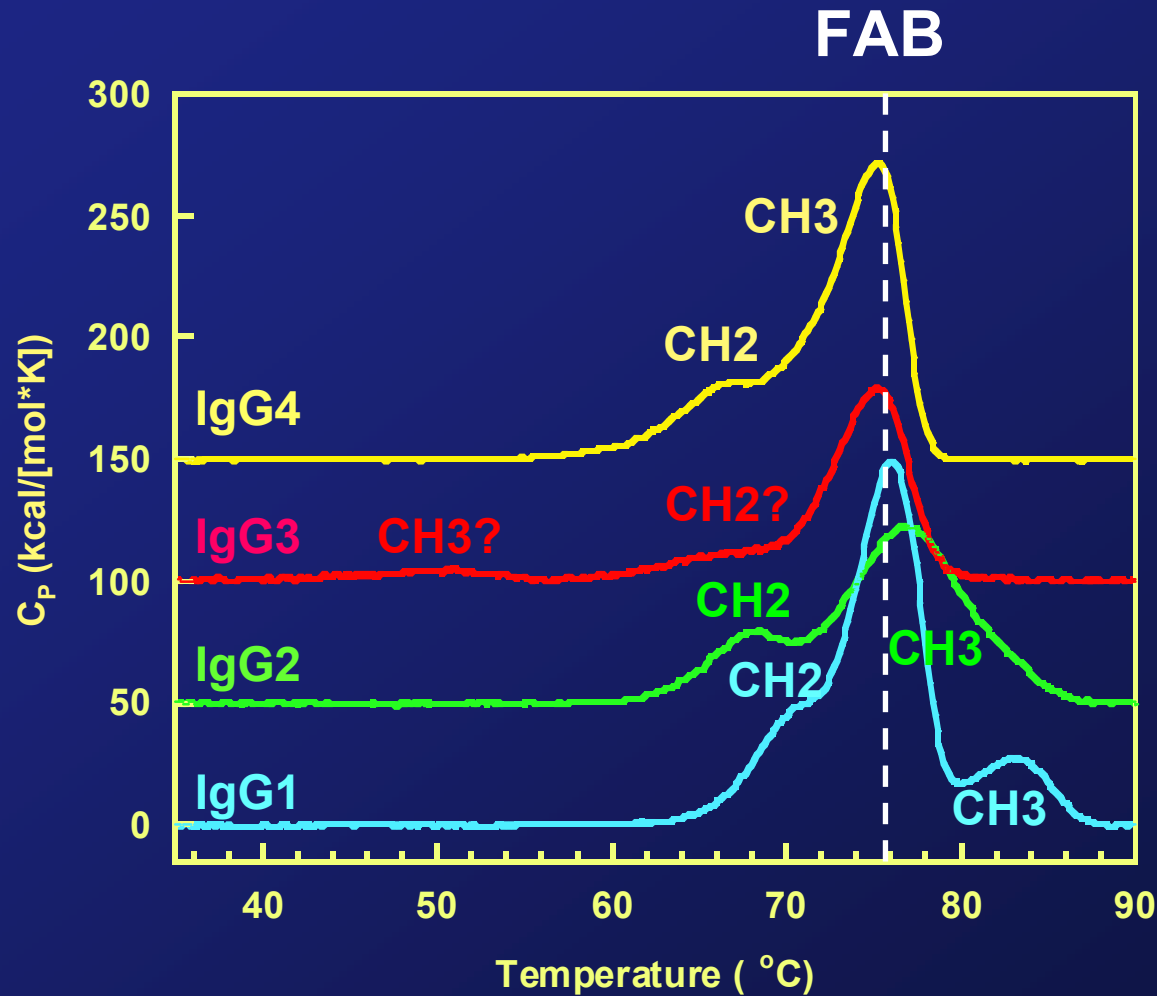
pH 4

pH 3.3

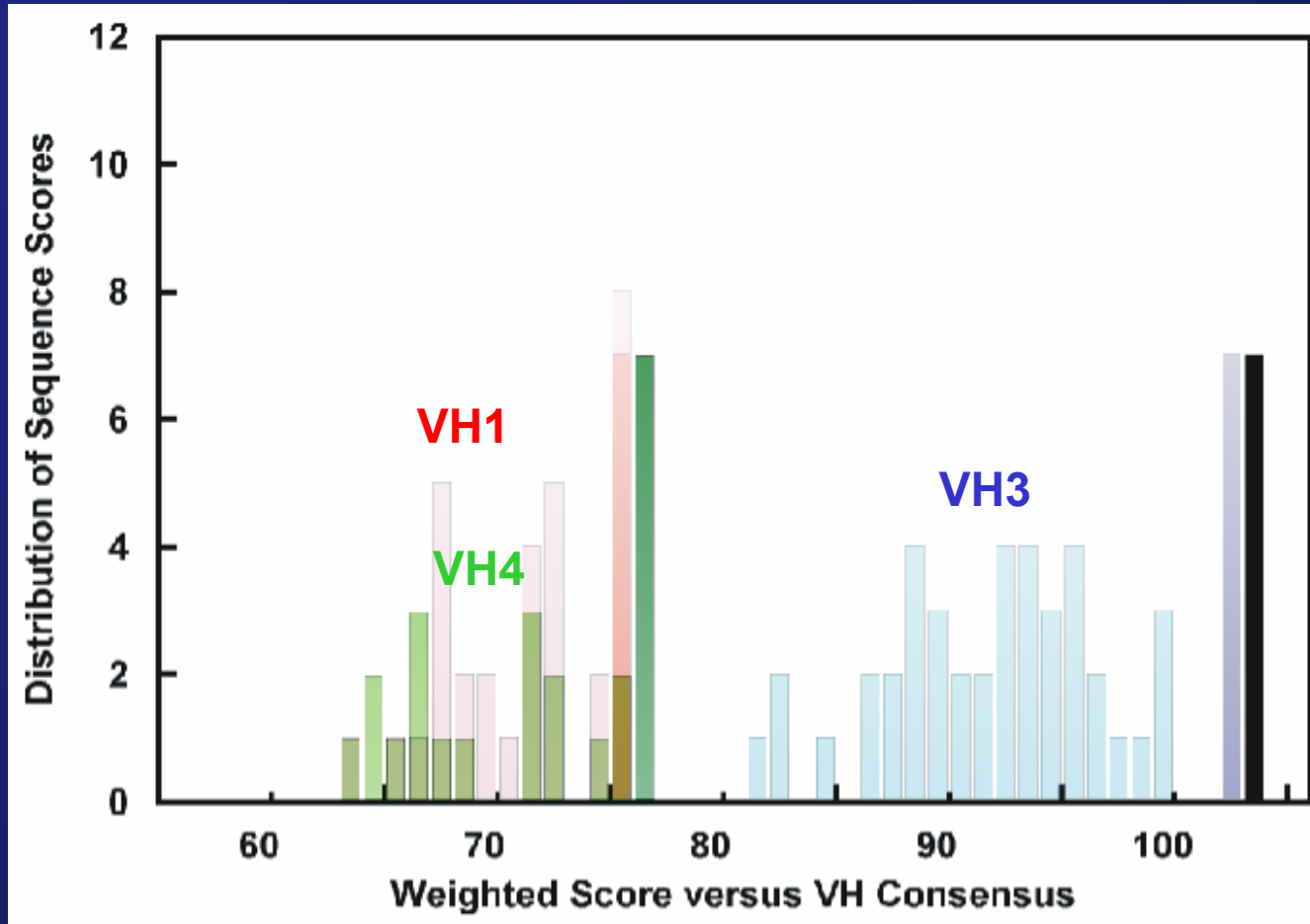
pH 2.8



IgG1 Has the Most Stable IgG-Fc

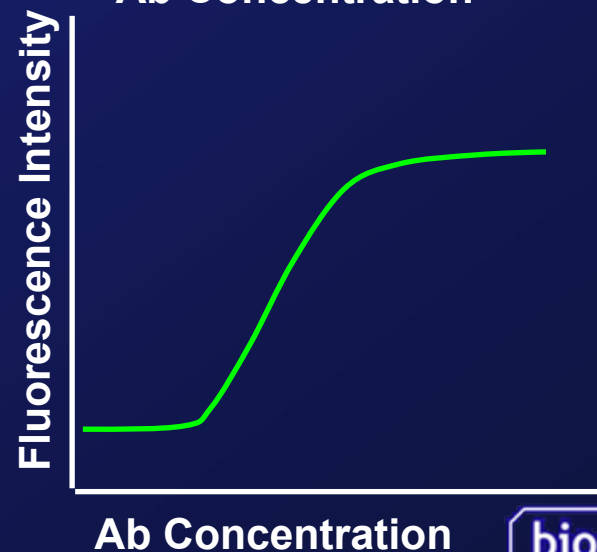
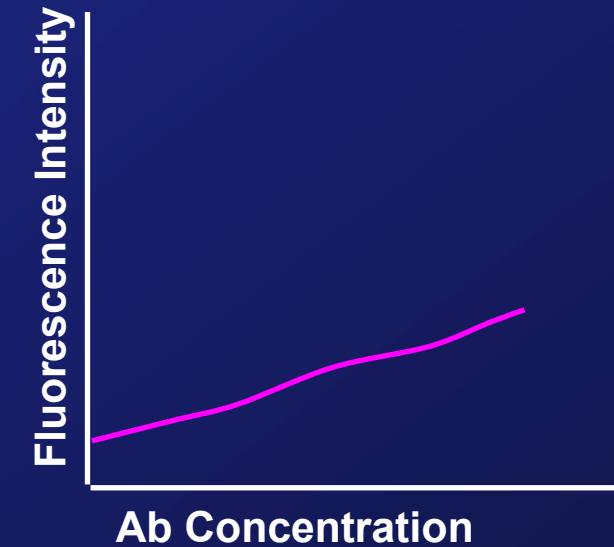
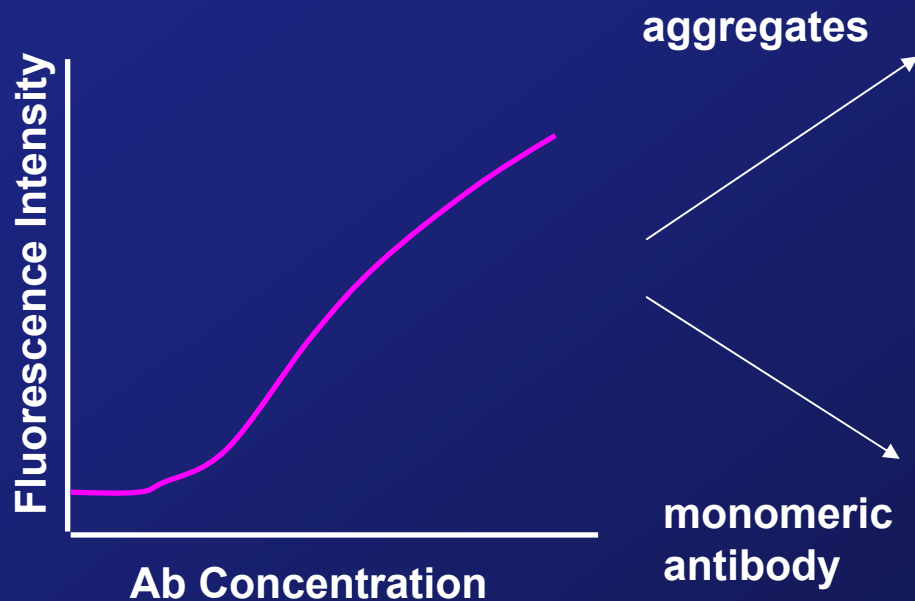


Distribution of *Human* VH Scores Against a Mammalian VH Database



**Scoring is Weighted*

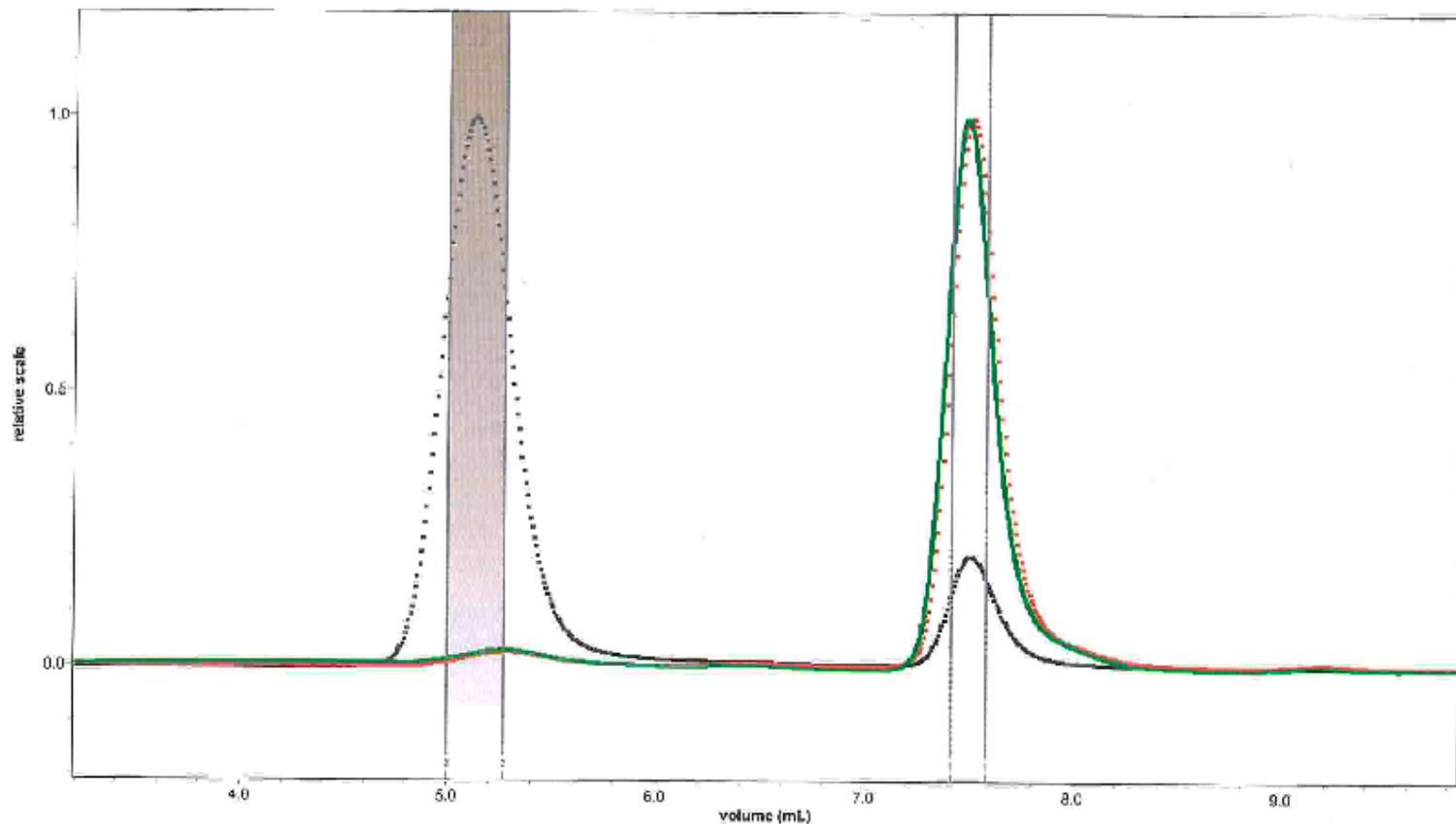
Aggregation/Instability Explains Anomalous FACS Behavior of **BIIB17**



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BIIB17 Instability/Aggregation Issues

Light Scattering Detects Formation of Aggregates



Variable domain Germline Subclasses

Based on genome and usage:

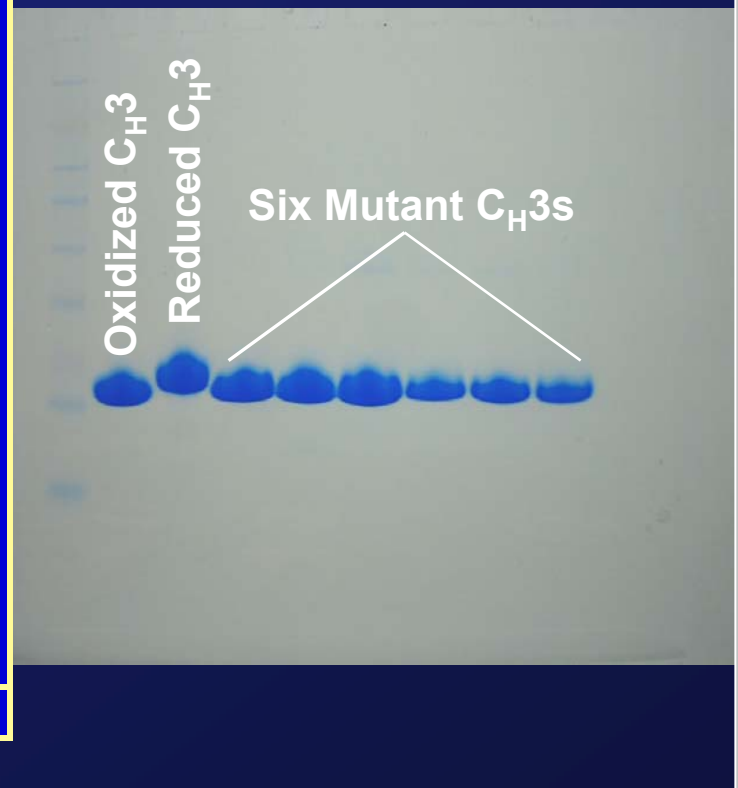
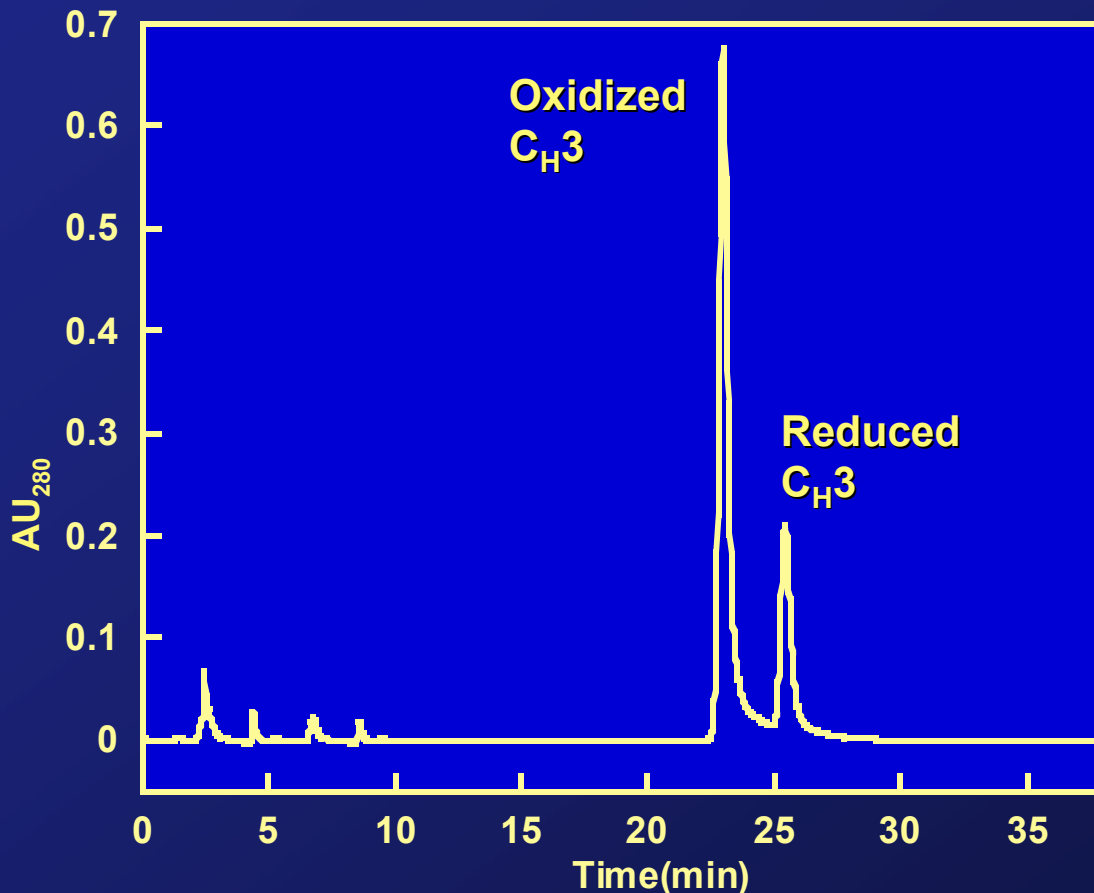
$$V_H = V_H1-V_H7$$

$$V_K = V_K1-4$$

$$V_\lambda = V_\lambda1-3$$

Overexpression of C_H3 Domains in Bacteria

- Cytoplasmic production of oxidized C_H3 using BL21trxB(DE3).

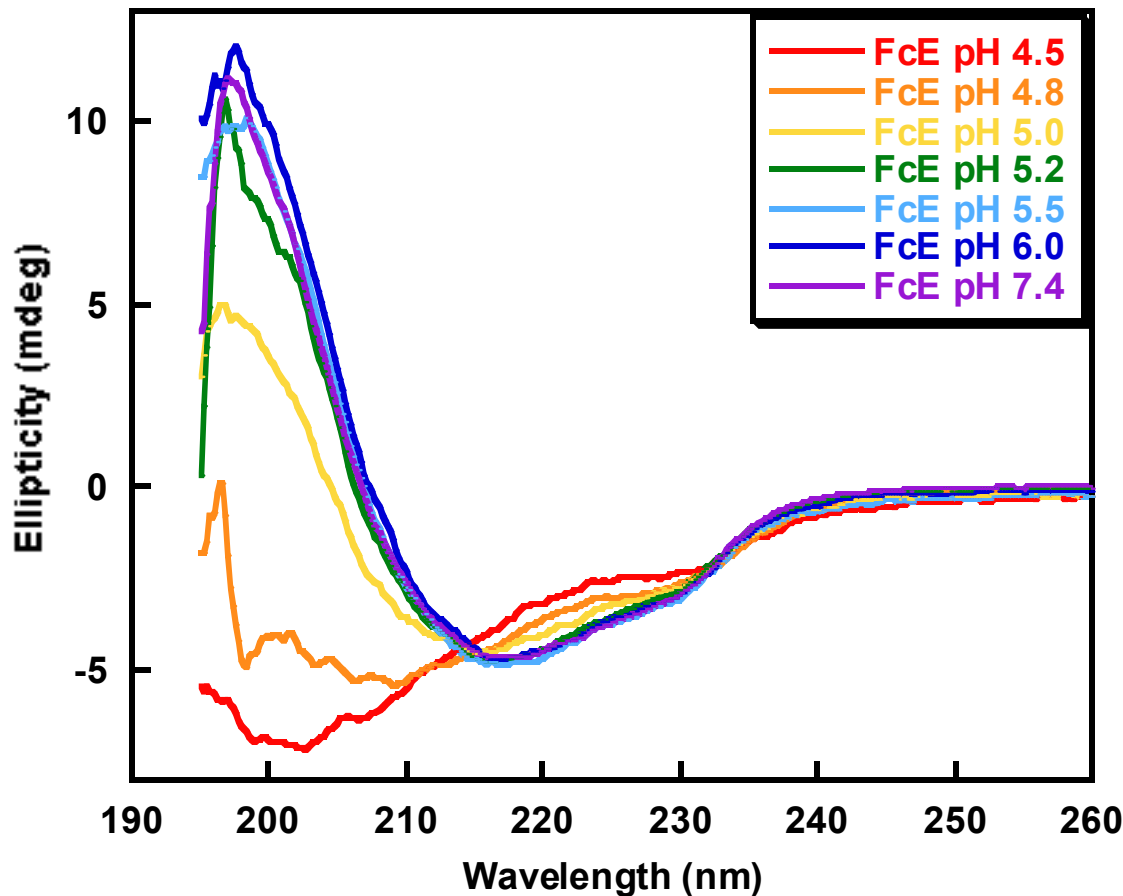


Outline

1. Sequence-Based Engineering and Prediction of Antibody Domain Stability
2. Variations in Stability between Antibody Isotypes
 - IgG vs. IgE

FcE Undergoes a Structural Change < pH 5

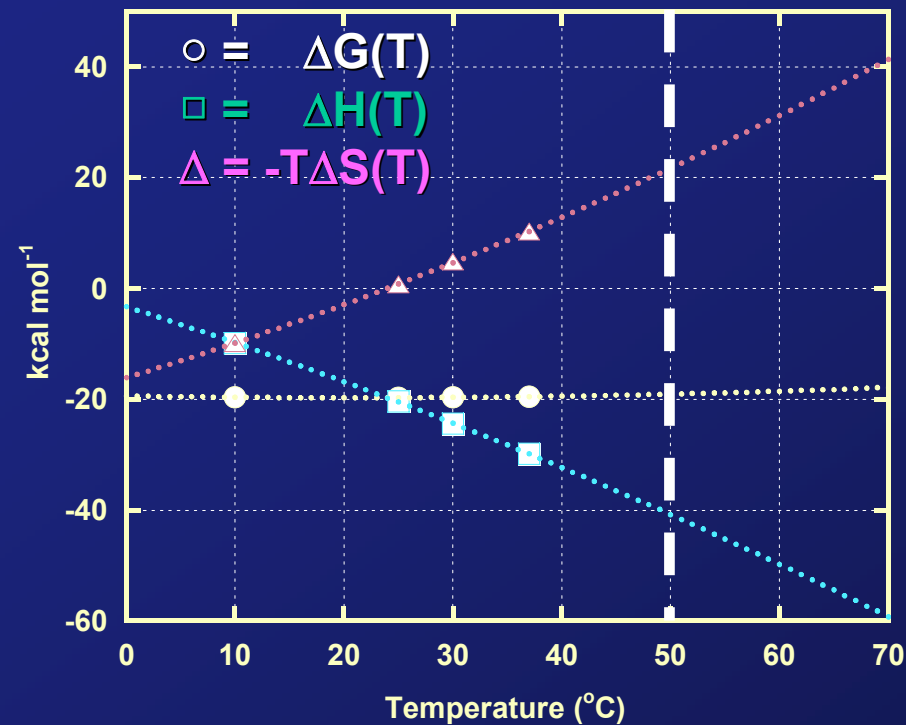
Circular Dichroism (CD)
Spectra of FcE



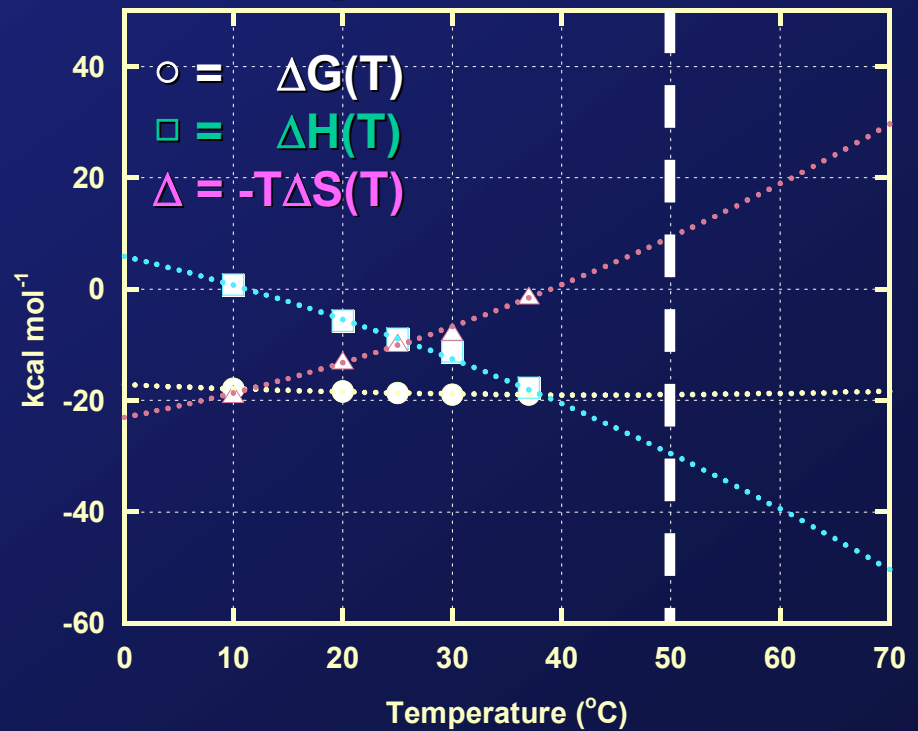
- FcE structural transition < pH 5 coincides with SEC/ANS data
- FcE structure is identical at pHs 5-9

pH Dependent Thermodynamic Changes in the FcE/FcERI Interaction

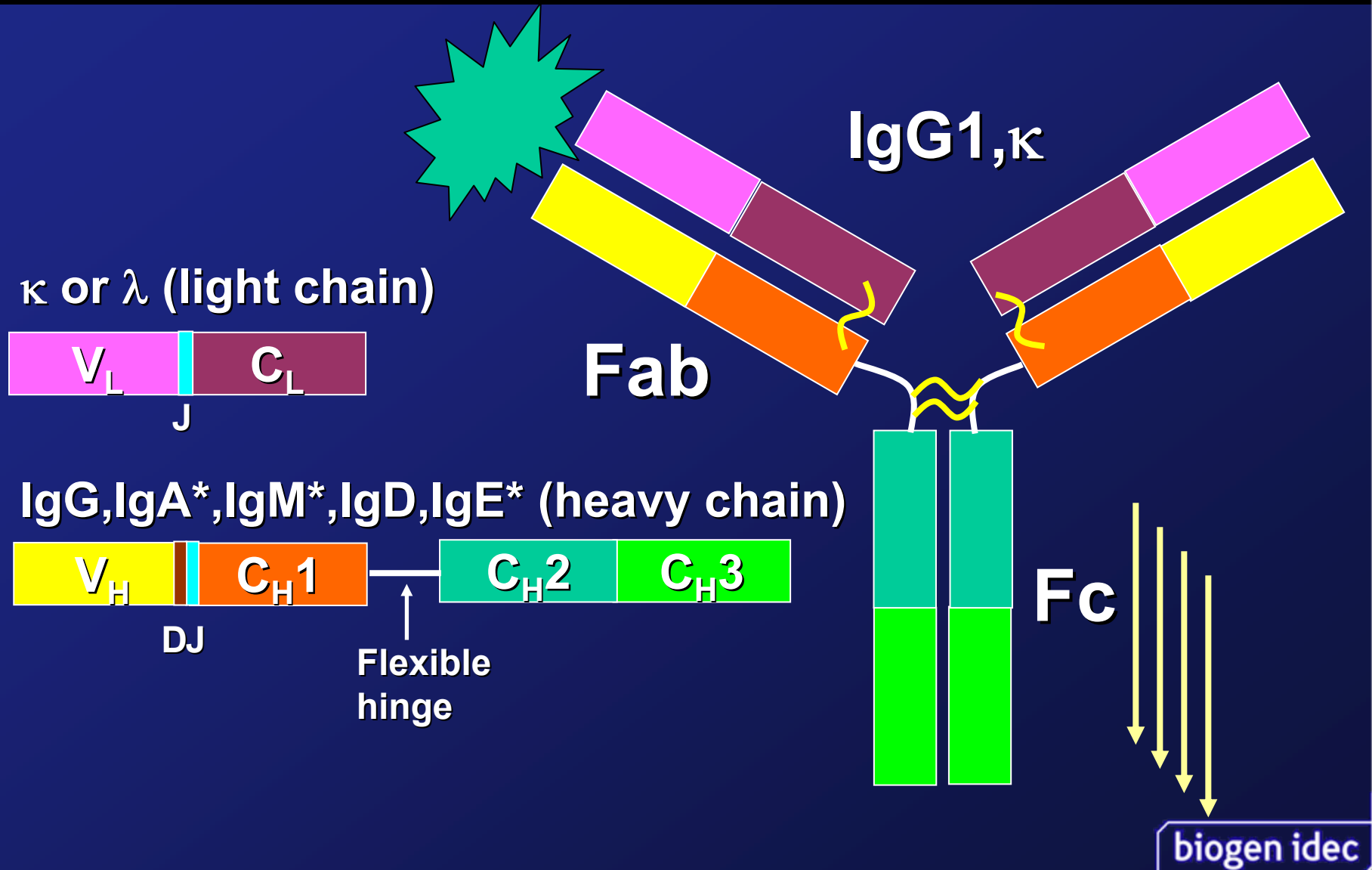
pH 7.4



pH 6.0



Schematic Diagram of an Antibody



Equations:

$$K_{eq}(T_M) = [FcERI_U][FcE_U]/[Complex] \text{ at } T_M.$$

Algebra:

$$(1) K_A(T_M) = K_U FcE(T_M) * K_U FcERI(T_M) / K_{eq}(T_M)$$

Using the Gibbs-Helmholtz eqn:

$$(2) K_A(T_M) = (1/K_{eq}) * \exp[(-\Delta H(T_M \text{ apoFcE})/R) * (1/T_M - 1/T_M \text{ apoFcE}) + [\Delta C_p FcE/R] * (\ln(T_M/T_M \text{ apoFcE}) + (T_M \text{ apoFcE}/T_M) - 1) + [-\Delta H(T_M \text{ apoFcERI})/R] * (1/T_M - 1/T_M \text{ apoFcERI}) + [\Delta C_p FcERI/R] * (\ln(T_M/T_M \text{ apoFcERI}) + (T_M \text{ apoFcERI}/T_M) - 1)]$$

Once $K_A(T_M)$ is derived, extrapolate back to 25 °C using ΔC_p Complex and ΔH at 25 C, i.e.:

$$(3) K_A(25C) = 1/K_D(25C) = K_A(T_M) * \exp[(-\Delta H(25C)/R) * (1/T_{25C} - 1/T_M) + (\Delta C_p/R) * (\ln(T_{25C}/T_M) + 1 - (T_{25C}/T_M))]$$

Brandts, J.M and Lin L.-N. (1990) Biochemistry. 29, pp. 6927-6940.

How we get the Terms.....

K_A = Association Constant (1/M)

K_D = Dissociation Constant (M)

K_U FcE = Unfolding equilibrium constant of the FcE CE3-CE4 domains

K_U FcERI = Unfolding equilibrium constant of FcERI

$K_{eq}(T_M)$ = Equilibrium Constant for System at midpoint of Complex unfolding

T_M = Midpoint of the FcE/FcERI Complex melting curve

T_{M}^{apoFcE} = Midpoint of the free FcE CE3-CE4 domain simultaneous melting curve

T_{M}^{apoI}

ΔC_p^{FcI}

ΔC_p^{FcI}

ΔC_p^{Co}

$\Delta H(T_M)$

$\Delta H(T_M)$

$\Delta H(25^\circ C)$

R = Universal Gas Constant

$\Delta H(25^\circ C)$ = Enthalpy of complex formation

$\Delta C_p^{Complex}$ = Change in heat capacity upon complex formation

Blue = Derived from combination of experiments

Purple = Derived from a single experiment

Red = Assumption

Black = Constant

Green = Derived from Literature

Assumptions:.

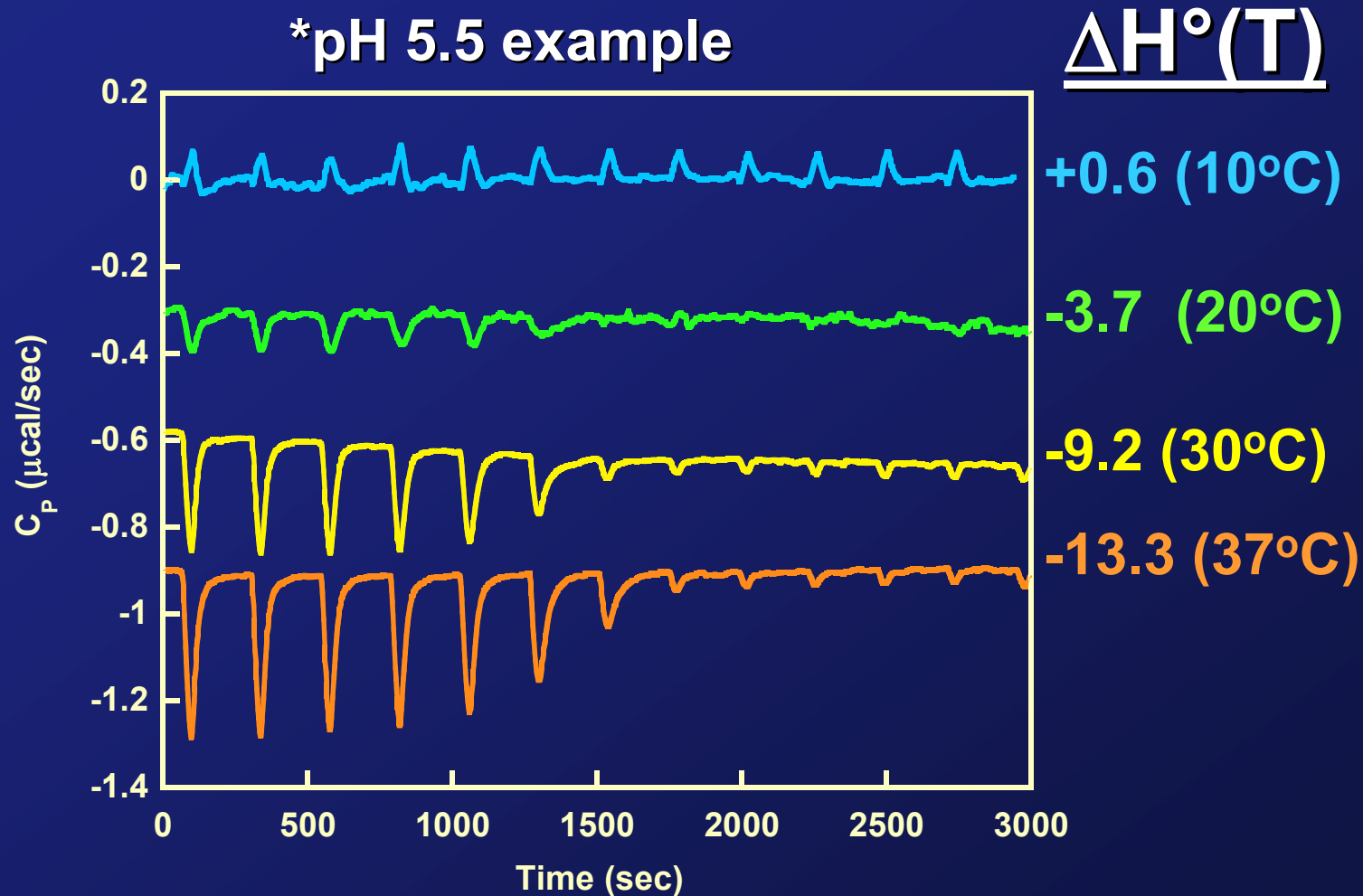
(1) ΔC_p^{FcE} and ΔC_p^{FcERI} are Estimated from the size of the two proteins.

(2) Cε2 and FcG are not part of the interaction.

(3) Two-State Unfolding (FAST scan rate)

$\Delta H^\circ(T)$ and ΔC_p° Terms for the Fc ϵ -Fc ϵ R1 α Interaction at Lower pHs

*pH 5.5 example

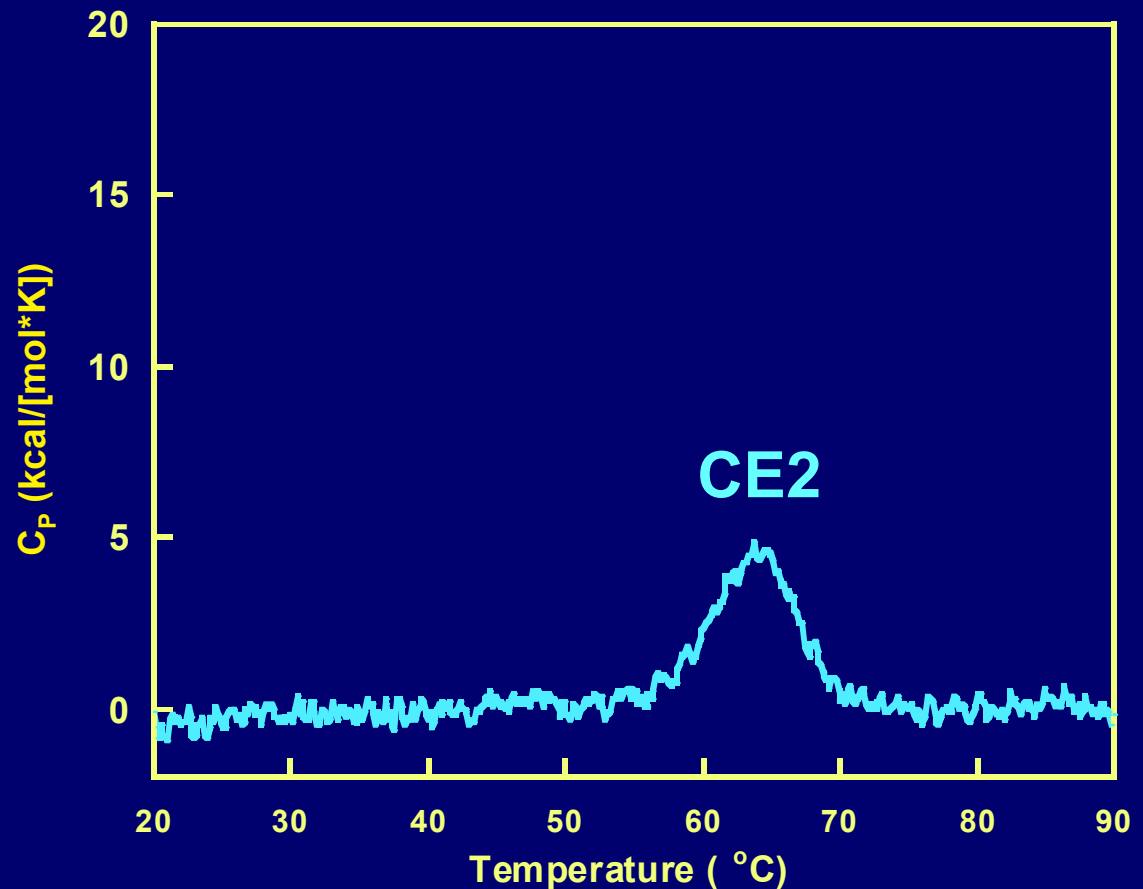


Introduction to Immunoglobulins

- **IgG** (IgG1, IgG2, IgG3, **IgG4**) - most common antibody isotype in serum used for host defense.
*****The Isotype of choice for Antibody Therapeutics!!**
- **IgE** - Wound healing and parasite defense. *Allergic disease.* *****New Classes of Antibody Therapeutics...**
- **IgA** (IgA1, IgA2, dIgA) - found in mucosal areas and secreted in the digestive tract to fight microorganisms.
- **IgM** - Pentameric, used as initial, low affinity immune response for clearing viral or bacterial agents.
- **IgD** - B-cell class switching, diseases and more...

FcE Unfolds Below pH 5

pH 7
pH 6
pH 5.5
pH 5
pH 4.8
pH 4.5
pH 3.5
pH 2.8



ΔC_p° at Low pH Values

